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Begin the article with a short summary.

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Clinical Pharmacology of Intrathecal Methotrexate. II. An Improved Dosage Regimen Derived From Age-Related Pharmacokinetics [1,2]

W. Archie Bleyer[3,4,*]

SUMMARY

Cerebrospinal fluid (CSF)-antifolate concentration was analyzed in 100 specimens from 47 patients treated with intrathecal methotrexate (MTX) (12 mg/m² of body surface area [BSA]). The drug concentrations varied 100-fold, with high levels associated with neurotoxicity and low levels with a poor response to therapy. CSF-MTX concentration was correlated directly with patient age, suggesting that a constant dose, regardless of age or BSA, should provide more consistent CSF-drug concentrations. In a subsequent study 25 patients treated with a conventional-dose schedule of 12 mg/m² of BSA were compared with a matched group of 24 patients administered a constant dose of 12 mg. There was significantly less variability of drug levels in the CSF with the constant-dose method than with the dosage derived from BSA. It is recommended that patients between 3 and 40 years of age receive the same intrathecal dose rather than varying doses adjusted for patient BSA.

[Cancer Treat Rep 61:1419–1425, 1977]

Although highly effective in most patients, intrathecal methotrexate (MTX) therapy is frequently associated with either an adverse neurologic reaction or an inadequate neoplastic response. In a previous report some of the clinical variability was related to the concentrations of MTX in the cerebrospinal fluid (CSF), although it was unclear as to why concentrations varied widely from patient to patient (1). In this study, the use of body surface area (BSA) for dosage determination was identified as a factor contributing to the variability. A constant dose irrespective of BSA was subsequently demonstrated to provide more consistent CSF-drug levels.

PATIENTS AND METHODS

Children were defined as patients ≤ 10 years of age, adolescents as patients 11–19 years of age, and adults as patients ≥ 20 years of age. Only those patients receiving six doses of intrathecal MTX at 2–8-day intervals for prevention or treatment of central nervous system (CNS) leukemia were included. Patients with lumbar-puncture syndrome, defined as orthostatic headache and/or low CSF pressure (< 20 mm), were excluded from analysis.

The initial observations were made in 47 patients with acute leukemia treated with intrathecal MTX via lumbar puncture at a dose of 12 mg/m² of BSA per dose. Absolute doses ranged from 4.8 to 29 mg. Twenty-five patients received prophylactic therapy and 22 were treated for overt meningeal leukemia, having malignant cells in the CSF. The patients ranged in age from 6 months to 64 years, and in BSA from 0.4 to 2.0 m². To correct CSF-MTX

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[2]Supported by Public Health Service grant CA-16525 from the National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare, and by a Junior Faculty Clinical Fellowship award from the American Cancer Society.

[3]Division of Hematology/Oncology, Children's Orthopedic Hospital and Medical Center, Department of Pediatrics, University of Washington, and the Fred Hutchinson Cancer Research Center, Seattle.

[4]I wish to gratefully acknowledge Jill L. Savitch for her technical help, Dr. Ross Prentice, Biostatistics, Fred Hutchinson Cancer Research Center, for his statistical analyses, and Drs. John S. Holcenberg, Milwaukee Children's Hospital, Brigid G. Leventhal, Johns Hopkins University Cancer Center, and Edward S. Henderson, Roswell Park Memorial Institute, for their assistance and advice.

*_Reprint requests to:_ W. Archie Bleyer, MD, Division of Hematology/Oncology, Children's Orthopedic Hospital and Medical Center, 4800 Sand Point Way NE, PO Box C-5371, Seattle, Wash 98105.

concentrations for variations in BSA relative to CNS volume, the following formula was used: (MTX concentration)(adult CNS volume)(patient CNS volume)$^{-1}$(mean adult dose)(patient dose)$^{-1}$. The CSF-drug level was corrected for CNS volume because the critical volume of distribution approximates the extracellular fluid volume of the CNS (1). The mean adult dose in this part of the study was 20 mg. The ratio of adult to patient CNS volume was derived from figure 1.

Subsequently, 49 children and adults receiving prophylactic intrathecal MTX for prevention of meningeal leukemia were treated with either the conventional-dose schedule of 12 mg/m²/dose or a constant dose of 12 mg. Twenty-five patients ranging in age from 3 to 26 years received a conventional schedule of 12 mg/m² of BSA, with absolute doses ranging from 6 to 28 mg. Twenty-four patients ranging in age from 3 to 39 years received a constant dose of 12 mg administered in a constant volume of 12 ml of Elliott's B solution.

In patients treated with doses based on BSA, the drug was diluted in Elliott's B solution to a concentration of 1.0–1.5 mg/ml and injected at a volume of 12 ml/m², with a maximum volume of 18 ml. There was no upper limit on the dose of MTX. BSA was derived from the patient's height and weight according to the West nomogram (2).

In general, CSF samples for MTX determination were obtained only at the time of lumbar puncture for the second and third scheduled doses of intrathecal chemotherapy. The interval between injections varied from 2 to 8 days depending on the clinical protocol. The number of CSF-MTX determinations on the day after injection were too few to include for the purpose of this study. Serial samples after single injections were not obtained except in patients who received a repeat lumbar puncture for evaluation of intrathecal MTX neurotoxicity. Since the residual MTX in the CSF 2 days after injection represented < 0.1% of the administered dose and since none of the patients demonstrated accumulation of the drug with excessive doses, concentrations measured after consecutive doses of MTX in the same patients were included in the analysis.

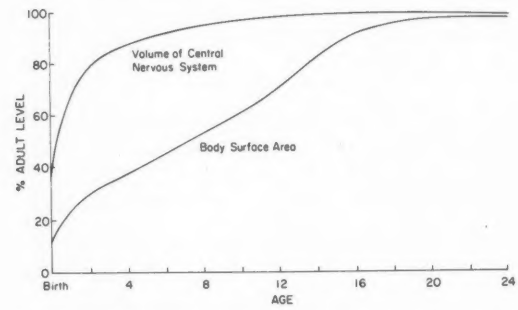

FIGURE 1.—Comparison of the mean values for CNS volume and BSA as a function of age. CNS volume was estimated from the combined weights of the brain, spinal cord, and CSF (6,7). BSA was derived from the West nomogram (2) using mean values for height and weight of children from the US.

Injections were performed by the same group of physicians utilizing the same technique throughout the study. Technical problems with drug administration were negligible. None of the initial drug administrations during the course of this study were considered to be complicated by inadvertent drug loss or extra-arachnoid injections. In all patients the solution was injected slowly without barbotage after isovolumetric removal of CSF.

One-half ml of CSF was kept in the dark at 4°C until antifolate measurement was performed, usually within 3 days after collection. MTX concentration was determined in triplicate by the dihydrofolate reductase inhibition method (3), with use of an enzyme derived from L1210 leukemic cells resistant to MTX (generously provided by Dr. David G. Johns, National Cancer Institute). The assay has a lower limit of sensitivity of 5×10^{-10} M in CSF.

RESULTS

Initial Observations

The first observation was that CSF-MTX concentration varied widely from patient to patient, ranging over a span of 2 to 3 logs (fig 2). The second observation was that ten patients with neurotoxic reactions to the drug had significantly higher antifolate levels in their CSF than patients without neurotoxicity (fig 2). Six of these patients developed severe meningismus, two sustained grand mal seizures, one had cerebellar dysfunction, and one died with quadriplegia. This finding confirms a preliminary report in a smaller group of patients relating neurotoxicity to elevated levels of MTX concentration in the CSF (1). A third observation was that the lower CSF-antifolate concentrations were observed in patients with the least therapeutic benefit from intrathecal chemotherapy (fig 2). One of these patients failed to achieve remission from overt meningeal leukemia and two others relapsed within 1 month after CNS therapy. There then seemed to be a zone of approximately 1 log between ineffective levels and toxic concentrations of the drug in the CSF.

The CSF-drug levels were then used in individual patients as a guide to dosage modification, decreas-

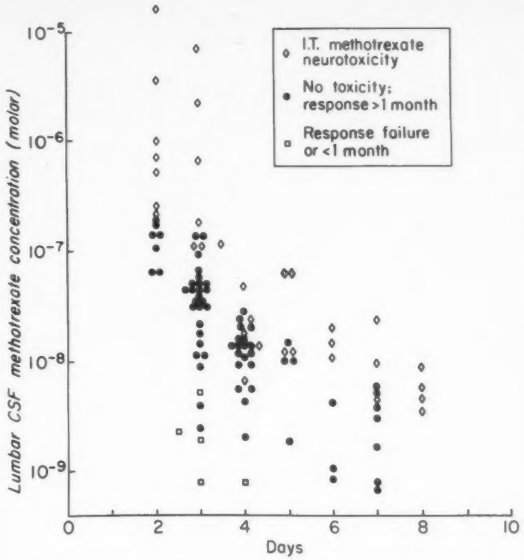
I.T. methotrexate neurotoxicity
No toxicity; response > 1 month
Response failure or < 1 month
Lumbar CSF methotrexate concentration (molar)
Days

FIGURE 2.—CSF-antifolate concentration after intrathecal MTX (12 mg/m² of BSA). ◇ = patients with neurotoxic reactions to intrathecal MTX, □ = patients who failed to achieve CNS remission or who had a meningeal relapse within 1 mo after therapy, and ● = patients who had neither neurotoxic reactions nor early CNS relapse.

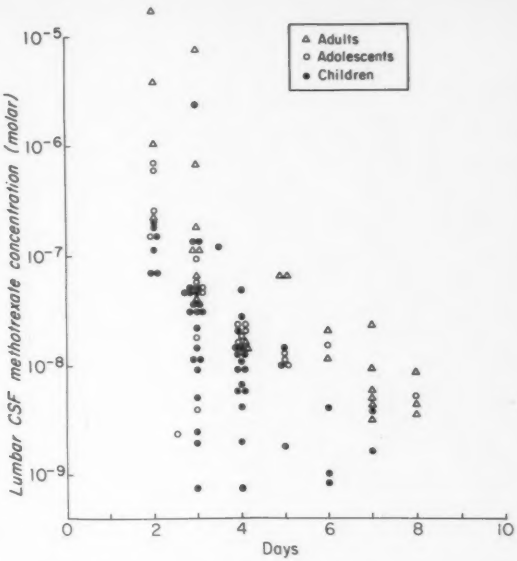

FIGURE 3.—CSF-antifolate concentration after intrathecal MTX (12 mg/m²). △ = patients ≥ 20 yrs, ○ = patients 11–19 yrs, and ● = patients ≤ 10 yrs.

ing the dose in patients with very high levels and increasing the dose in patients with very low levels. Several months after instituting this approach, it became apparent that the dosage was being decreased in adults and increased in young children. The two youngest children, for example, required doses of 20–25 mg/m² before the CSF-MTX concentration climbed into the "therapeutic zone." On the other hand, most adult patients required dose reductions into the range of 5–10 mg/m². This observation prompted a re-examination of the relationship between CSF-MTX concentration and patient age. It then became apparent that CSF-MTX concentrations were approximately proportional to age (fig 3). High values tended to occur in adults, intermediate values in adolescents, and low values in children. The linear regression curves of the log concentration values demonstrated these differences (fig 4A), and the F test indicated that the displacements of the regression curves along the concentration axis were significantly different ($F = 28.01$; 2 and 98 degrees of freedom; $P < 0.001$). The slopes were not significantly different ($F = 3.54$). When corrected for CNS volume, the regression curve for children coincided with the curve for adolescents, but both of these curves remained below the regression line for adult patients (fig 4B).

A comparison of the three age groups (table 1) failed to reveal any consistent differences in sex, simultaneous irradiation of the CNS, CSF protein concentration, CSF neutrophil count, or renal function as assessed by serum creatinine. Proportionately more adults (80%) were treated for overt meningeal leukemia than were adolescents and children combined (46%), but this difference and the overall trend and differences between individual groups were not significant. The incidence of MTX-induced neurotoxicity was directly proportional to patient age, with one of 29 children (3%), three of eight adolescents (38%), and six of ten adults (60%) having developed this complication ($X^2 = 15.71$; $P < 0.0005$).

Serial CSF samples after single injections were obtained seven times in five patients with intrathecal MTX neurotoxicity. In general, the curves were higher than and parallel to the age-specific regression lines. Within the group, however, there appeared to be a correlation of the half-disappearance time with CNS leukemia: the CSF-MTX half-life was 12, 12, and 16 hours during prophylactic ther-

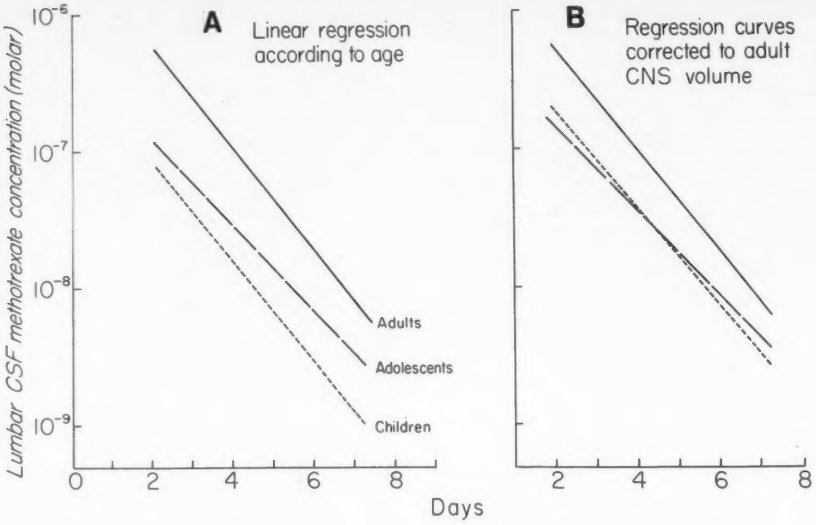

FIGURE 4.—A. Linear regression curves of the log values shown in fig 3. B. Same curves in A corrected to adult CNS volume according to the formula given in the text under Methods.

apy and 18, 19, 21, 24, and 30 hours during treatment for overt disease.

Because overt meningeal leukemia occurred more commonly in adults than in younger patients, CSF-MTX concentrations were examined in patients treated prophylactically separately from those treated for established CNS disease. Regression analysis was not possible because of insufficient number of drug determinations in each of the subgroups. Alternatively, the number of patients in each age bracket above and below the geometric mean regression curve for each treatment group were compared by X^2 analyses. A direct correlation of CSF-MTX concentration with age was observed in both treatment groups (table 2). The correlation was greater for treatment ($P < 0.001$) than for prophylaxis ($0.01 < P < 0.02$).

A similar analysis was performed for the effects of prophylactic versus active treatment within each age group, using the age-specific geometric mean regression curves shown in figure 4A. In each age bracket there were more CSF-MTX values above the regression curve in patients treated for overt disease than in patients treated prophylactically

TABLE 1.—Comparison of the 3 age groups

Data compared	Children (≤ 10 yrs)	Adolescents (11–19 yrs)	Adults (≥ 20 yrs)
No. of patients	29	8	10
BSA (m^2)*	0.8(0.4–1.0)	1.3(1.1–1.8)	1.7(1.5–2.0)
Male patients	16	5	5
Patients also treated with cranial irradiation	12	2	3
CSF neutrophils (μl^{-1})*	2(0–10)	9(0–20)	11(0–26)
CSF protein (mg/dl)*	37(20–85)	25(15–50)	35(19–113)
Serum creatinine (mg/dl)*	0.6(0.2–1.3)	0.8(0.2–1.5)	0.7(0.5–1.3)
Patients with overt meningeal leukemia	15	2	8
Patients with MTX neurotoxicity	1	3	6

*Values are median and range.

TABLE 2.—CSF-MTX concentration as a function of age within treatment groups

Treatment group	No. of patients	No. of CSF-MTX levels— Below geometric mean	Above geometric mean	
Prophylaxis				
Children	14	16	12	$\chi^2 = 8.20$
Adolescents	6	3	10	$P < 0.02$
Adults	2	0	5	
Treatment				
Children	15	21	7	$\chi^2 = 15.16$
Adolescents	2	4	5	$P < 0.001$
Adults	8	4	17	

TABLE 3.—CSF-MTX concentration as a function of disease status within age groups

Age group	No. of patients	No. of CSF-MTX levels— Below geometric mean	Above geometric mean	
Children				
Prophylaxis	14	17	12	$\chi^2 = 7.11$
Treatment	15	6	20	$P < 0.01$
Adolescents				
Prophylaxis	6	6	4	$\chi^2 = 2.99$
Treatment	2	1	8	$P > 0.05$
Adults				
Prophylaxis	2	3	3	$\chi^2 = 0.08$
Treatment	8	7	14	$P >> 0.05$

TABLE 4.—Comparison of clinical parameters in patients administered intrathecal dose based on BSA and patients treated with a constant dose

Data compared	Intrathecal MTX 12 mg/m²	12 mg
No. of patients	25	24
Median age in yrs (range)	11 (3–26)	10 (3–39)
Median BSA in m² (range)	1.0 (0.6–2.3)	0.9 (0.6–2.1)
Male patients	12	13
Patients treated concurrently with cranial irradiation	23	22
Mean absolute MTX dose in mg (range)	12 (7–28)	12 (—)

(table 3). The differences were significant only for children, however, possibly because of the smaller number of patients studied in the older age groups.

Constant Dose Versus BSA Dose

This study compared the conventional-dose method (12 mg/m² of BSA) with a constant dose of 12 mg regardless of age or BSA. The two treatment groups were comparable in age, sex, BSA, and concurrent cranial irradiation (table 4). CSF-MTX concentrations for the two treatment groups are shown in figures 5 and 6, with the solid lines representing the linear regression curves of log values and the dashed curves representing the 95% confidence interval of the true regression line (ie, the probability that the true regression line lies

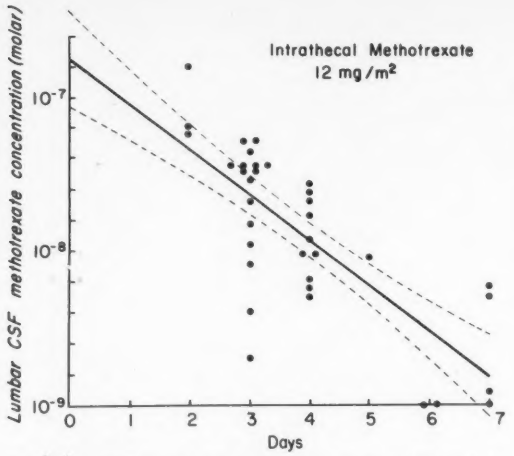

FIGURE 5.—CSF-antifolate concentrations in 25 patients administered MTX (12 mg/m² of BSA) via lumbar puncture. The solid and dashed curves represent the linear regression line and the 95% confidence range respectively.

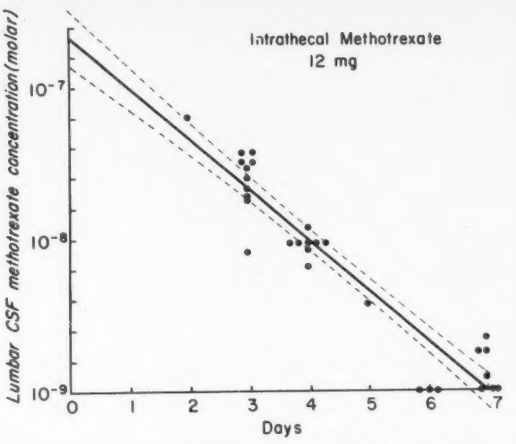

FIGURE 6.—CSF-antifolate concentrations in 24 patients administered the same absolute dose of MTX (12 mg) via lumbar puncture. The solid and dashed curves represent the linear regression line and the 95% confidence range respectively.

within the interval is 0.95). The group of patients administered a constant dose (fig 5) had a confidence interval that was significantly narrower than that observed in the group of patients treated with doses based on BSA (fig 6) ($F = 3.84$; 62 and 96 degrees of freedom; $P < 0.01$). The slopes of the linear regression curves were essentially identical.

The geometric mean (± 1 SD) molar concentrations for 3, 4, and 7 days after injection were 2.8 (± 1.60) × 10^{-8} M, 1.4(± 0.80) × 10^{-8} M, and 3.2(± 2.50) × 10^{-9} M respectively for the 12-mg/m² dose group, and 2.5(± 0.95) × 10^{-8} M, 1.0(± 0.15) × 10^{-8} M, and 1.5(± 0.50) × 10^{-9} M respectively for the 12-mg dose group. This comparison reveals that the SDs were 1.7–5.3 times greater in the group whose dosage was based on BSA than in the constant-dose group.

DISCUSSION

This study indicates that with dosage based on BSA, CSF-MTX concentrations 2–8 days after intrathecal administration are directly proportional to patient age. This finding may be explained by postulating that older patients may have a slower rate of disappearance of MTX from lumbar CSF, although this hypothesis does not seem likely in view of the similar rates of disappearance observed for all three age ranges (fig 4). A more satisfactory explanation is that older patients receive a greater amount of drug relative to the drug's volume of distribution. An increased ratio of drug dose to distribution volume may be due either to a decreased volume of distribution or to a relative increase in the absolute dose. Since there is no evidence that the interstitial, extracellular, or intracellular volumes of the CNS decrease with age during the first 60 years of life, the age correlation is probably due primarily to the administration of a relatively greater dose in older patients. This phenomenon is most likely a consequence of the use of BSA for intrathecal dosage calculation.

BSA appears to be an inappropriate basis on which to calculate intrathecal drug dosage. After all, the growth of the CNS does not parallel changes in BSA, and if the drug distributes primarily within the CNS, as does MTX (4), its concentration × time profile should depend more on CNS parameters than on BSA. CNS volume reaches adult levels within a few years, whereas BSA continues to increase throughout childhood and adolescence (fig 1). Thus, the volume of distribution is relatively independent of age after the first 3 years of life and dosage based on BSA would be expected to result in low concentrations in young children and relatively higher concentrations in older persons, as observed in this study.

A previous study suggested that administration of intrathecal MTX to patients with overt meningeal leukemia may result in elevated CSF-antifolate concentrations (1). This trend was also observed in the current study. To clarify the relative contributions of age and leukemic meningitis to the disappearance kinetics, the three age groups were compared among those patients treated prophylactically separately from those treated for established me-

ningeal leukemia. A direct correlation of CSF-MTX concentration with age was observed in both treatment groups, indicating that although the presence of overt meningeal leukemia may have contributed to the apparent age correlation, it could not have accounted for the age-related differences observed. The reverse analysis, comparing the two treatments in each of the three age groups, indicates that age and disease act independently as factors contributing to the variability of CSF-MTX concentrations. It was difficult to quantitate the relative contributions because of the small number of drug determinations in several of the subgroups. There was a suggestion, however, that overt CNS leukemia may result in higher CSF-drug levels than increasing age because the age correlation was greater in patients treated for overt disease than in patients treated prophylactically.

A higher incidence of neurotoxicity associated with intrathecal MTX was observed in adolescents and adults, as previously reported (1,5). On the other hand, very low antifolate concentrations were associated with an inadequate neoplastic response. It would seem prudent then to develop dosage regimens that produce more consistent CSF-drug concentrations.

One approach suggested by this study is to adjust intrathecal dosage in relation to a body parameter that reflects the intracranial volume, such as head circumference. Another approach would be to administer a constant dose regardless of age or BSA in patients >3 years of age, as suggested by the constancy of CNS volume after the first few years of life (fig 1). Indeed, when the age-specific regression curves were corrected for CNS volume, the curves for children and adolescents coincided. The fact that these curves did not coincide with the adult curve is probably due to the proportionately larger number of adult patients treated for overt CNS leukemia.

In the second study reported here, the constant-dose approach was compared with the conventional-dose method in patients receiving prophylactic intrathecal MTX for acute leukemia. The results indicated that the mean values for CSF-MTX concentration in the two treatment groups were comparable. The similarity is probably due to the fact that the median BSA in the conventional-dose group was 1.0 mg/m^2. Hence, the mean absolute dose administered to this group was 12 mg, the same dose given to all patients in the constant-dose group.

There was, however, significantly less variability of drug levels with the constant-dose method than with the BSA approach. This difference may be related, at least in part, to the constancy of the drug's volume of distribution within the CNS relative to the steady increase in BSA with age. Since clinical effect appears to be related to CSF-MTX concentration, the constant-dose method appears to represent improvement in intrathecal chemotherapy.

The optimal drug dosage in patients <3 years of age is not known, however, since children of this age were not included in the comparative dose study. Dosage should probably be adjusted downward in very young patients to compensate for a reduced CNS volume (fig 1). Until additional data are available, 6 mg for children <1 year of age, 8 mg for children between 1 and 2 years of age, and 10 mg for 2 year olds are suggested, dosage increments which parallel the changes in CNS volume during the first 3 years of life (fig 1). This dosage schedule has been used thus far in six infants ranging in age from 6 to 34 months. None have developed signs of intrathecal MTX neurotoxicity.

The optimal dosage for elderly patients also cannot be defined from this study because the oldest patient in the comparative dose study was 39 years of age. Patients >60 years of age may require lower doses, however, since the volume of the brain begins to decrease after the age of 60 (6) and because CSF turnover may also be diminished in patients of advanced age.

In conclusion, variability in CSF concentrations can be significantly decreased in patients between 3 and 40 years of age if they receive the same intrathecal dose rather than varying doses adjusted for patient BSA. This concept is likely to apply to any intrathecally administered drug that distributes primarily within the CNS.

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Role of Radiation Therapy in Combination With Chemotherapy in Extensive Oat Cell Cancer of the Lung: A Randomized Study [1,2]

Christopher Williams, Michael Alexander, Eli J. Glatstein, and John R. Daniels [3,*]

SUMMARY

After 3 months of chemotherapy with procarbazine, vincristine, cyclophosphamide, and CCNU, patients with extensive oat cell carcinoma of the lung were randomly allocated to receive 3000 rads of involved-field irradiation and further chemotherapy or to continue with chemotherapy only. In addition, all patients received 3000 rads of prophylactic whole-brain irradiation. The addition of involved-field radiation, as given in this trial, not only failed to improve survival but was unable to alter the pattern of sites of initial relapse. There were no cases of extension to the brain in 31 consecutive patients who received prophylactic whole-brain irradiation. This was in strong contrast to a preceding trial in which six cases of brain extension occurred among 19 patients who did not receive prophylactic whole-brain irradiation.

[Cancer Treat Rep 61:1427–1431, 1977]

Earlier clinical trials in oat cell carcinoma at Stanford (1) suggested the superiority of a four-drug program consisting of procarbazine, vincristine, cyclophosphamide, and CCNU (POCC) over a three-drug schedule consisting of cyclophosphamide, vincristine, and methotrexate (COM) in the setting of involved-field radiation therapy sandwiched after two or three cycles of chemotherapy. Analysis of patterns of relapse revealed a high incidence of brain extension and frequent lack of long-term control in the irradiated sites.

In this study, the value of involved-field radiation (3000 rads in 2 weeks) has been tested by a randomized trial in patients with nonlocalized disease. All patients received prophylactic whole-brain radiation and are compared with our recent historical controls for incidence of brain extension.

MATERIALS AND METHODS

Twenty-seven patients were entered in this study between January 1975 and September 1976. The criteria for admission were:

(*a*) biopsy or sputum cytology demonstrating unequivocal oat cell carcinoma (WHO classification [2] small cell anaplastic, lymphocyte type);
(*b*) previously untreated with radiotherapy or chemotherapy;
(*c*) Karnofsky status (3) of ≥ 20;
(*d*) no other malignant disease in the preceding 5 years apart from basal cell carcinoma of the skin;
(*e*) informed consent;
(*f*) age ≤ 70 years;
(*g*) advanced disease: stage T = 3, or N = 3, or M = 1.

Staging was based on the International Union Against Cancer system as modified by the Radiation Oncology Group (4).

Initial evaluation in all patients included chest roentgenogram, bone scan, posterior iliac crest bone marrow biopsy, liver scan, brain scan or computer-axial tomogram (CAT) of the brain, chemical screen, and complete blood cell and platelet counts.

The treatment schema is presented in figure 1.

All patients received POCC chemotherapy and whole-brain irradiation. POCC chemotherapy con-

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[3]Divisions of Medical Oncology and Radiation Therapy, Stanford University School of Medicine, Calif.

**Reprint requests to:* John R. Daniels, MD, S-025, Division of Oncology, Stanford University Hospital, Stanford, Calif 94305.

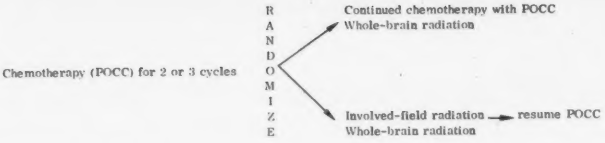

FIGURE 1.—Treatment schema.

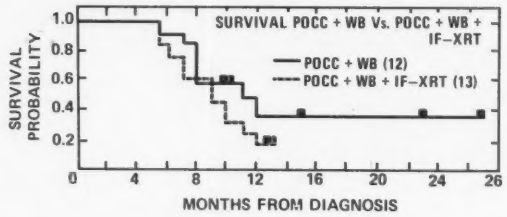

FIGURE 2.—Actuarial survival curves. WB = whole-brain radiation; IF-XRT = involved-field radiation.

sists of procarbazine, 100 mg/m² orally on Days 2–15; vincristine, 2 mg iv on Days 1 and 8; cyclophosphamide, 600 mg/m² iv on Days 1 and 8; and CCNU, 60 mg/m² orally on Day 1. Cycles are repeated at 28-day intervals. Doses of cyclophosphamide, CCNU, and procarbazine were adjusted for hematologic toxicity, and vincristine for neurotoxicity as previously described (1).

After two to three cycles of POCC, chemotherapy was interrupted in the half of the patients randomized to receive involved-field radiation to all known sites of disease except the bone marrow. Following radiation therapy, POCC chemotherapy was reinstituted.

Radiotherapy was delivered by a 4-MeV linear accelerator. All patients received prophylactic irradiation to the whole brain after two to three cycles of POCC, a 3000-rad mid-plane dose being delivered over 2 weeks via opposed lateral fields. For those patients randomized to involved-field radiation, chest disease was treated with 3000 rads given in 2 weeks to the primary tumor and a hilar, mediastinal, and supraclavicular port by an anterior-posterior opposing-field technique. Hepatic involvement was treated with 2500 rads in 3 weeks by an anterior-posterior opposed technique and pleural masses or effusions received 1500–2000 rads to the hemithorax over 2 or 3 weeks. The chest and brain were usually treated simultaneously; other major sites were treated in sequence.

Patients in either arm of the study were permitted to receive emergency radiation before or during the first course of POCC if respiratory or CNS symptoms required immediate relief. Following completion of radiotherapy, it had been planned to continue chemotherapy until tumor progression or until 2 years of disease-free survival had elapsed since the initiation of therapy.

Responses were scored as complete (CR), partial (PR), or no response. CR required disappearance of all measurable disease including a return to normal of all previously positive tests. PR required > 50% regression in the diameter of all measurable lesions, with no new lesions appearing. All lesser responses were classified as no response. The duration of response required for classification as CR or PR was at least 1 month.

RESULTS

Patient Characteristics

Comparison of patients in each arm (table 1) shows the two groups to be similar in age, Karnofsky status, and stage. However, there was a predominance of men in the involved-field radiation group. Distribution and frequency of metastatic disease were similar in both groups. One patient from each arm was unevaluable because of inappropriate use or omission of radiation therapy. These patients were excluded from analysis.

Survival

Actuarial survival curves are presented in figure 2. Median survival for the entire group is 10 months. Median survival for the POCC and whole-brain radiation group is 11 months, compared with 9 months for those receiving POCC, whole-brain radiation, and involved-field radiation.

Prior to radiation, the proportion of patients achieving CR appeared superior in the involved-field radiation group. However, following involved-field radiation no patients in that group had an improvement in overall remission status while five patients in the POCC group had continued improvement of both no responses and PRs, resulting in a similar distribution of responses (table 2).

The initial sites of relapse in those patients achieving a response are similar in both arms and were always in a site which had been involved at

TABLE 1.—Patient characteristics

	POCC + whole-brain radiation	POCC + involved-field radiation + whole-brain radiation
Total No. of patients	12	13
Mean age in yrs (range)	54.8 (31–65)	56.2 (46–68)
Sex		
Men	5	9
Women	7	4
Stage		
T_3	8	9
$\geq N_3$	6	5
M_1	5	6
Involved site		
Bone marrow	3	2
Bone scan	3	2
Liver	3	3
CNS	0	0
Karnofsky status		
80–100	7	7
50–80	2	4
20–50	4	3

presentation (table 3). Two patients who had achieved marginal responses to POCC (no response) had progression in the radiation field during involved-field radiation. One patient in the POCC group received 5000 rads of emergency mediastinal radiation for superior vena cava syndrome. This patient is the only long-term survivor (27+ months) and has not been receiving any chemotherapy for 13 months. Treatment was discontinued in this patient because of progressive treatment-associated disability and an apparent organic mental status change which reversed with cessation of chemotherapy. Three patients received radiation to the liver; one failed in that organ as the initial site of relapse and the other two ultimately manifested recurrent intrahepatic tumor during the course of their disease.

The administration of involved-field radiation delayed the reinstitution of POCC chemotherapy. The mean time interval between the completion of the POCC cycle prior to involved-field radiation and the start of the POCC cycle following involved-field radiation was 66 days (range, 28–92 days), whereas the mean time interval between corresponding

TABLE 2.—Response to POCC chemotherapy

	No. of responses					
	Prior to radiation therapy			After radiation therapy		
	CR	PR	None	CR	PR	None
POCC + whole-brain radiation (12 patients)	2	7	3	5	6	1
POCC + whole-brain radiation + involved-field radiation (13 patients)	5	4	4	5	4	4

TABLE 3.—Incidence of initial relapse in site involved at presentation

Involved site	POCC + whole-brain radiation*		POCC + involved-field radiation + whole-brain radiation†	
	At relapse	At presentation	At relapse	At presentation
Chest	7	7	8	7
Bone	3	2	3	3‡
Liver	0	0	1	1

*1 patient had disease involving bone and chest.
†2 patients had disease involving bone and chest and 1 patient had involvement of liver and chest.
‡2 patients had bone marrow disease and did not receive radiation to bone.

TABLE 4.—Dose reductions

Drug	Time of reduction	% of calculated dose given	
		POCC	POCC + involved-field radiation
Procarbazine	Before radiation*	64	65
	After radiation†	41	55
Vincristine	Before radiation	72	77
	After radiation	56	41
CCNU	Before radiation	85	92
	After radiation	60	80
Cyclophosphamide	Before radiation	72	73
	After radiation	45	61

*POCC cycles 1–3.
†POCC cycles 4–9.

cycles in the POCC plus whole-brain irradiation arm was 30 days (range, 18–70 days). Required dose reductions were similar in both arms (table 4) although the radiotherapy patients received slightly higher doses after radiation.

Eight of 12 patients in the involved-field radiation arm had progression of disease within the first two cycles of POCC following radiotherapy. In the POCC arm, four of 11 patients relapsed during the corresponding time.

Whole-Brain Irradiation

In the preceding oat cell study at this institution (1) there were six cases of CNS relapse among 19 patients who had not been treated with prophylactic whole-brain irradiation. The last four patients entered in that trial received prophylactic whole-brain irradiation and none of these nor any of the 27 patients entered into the current study has developed CNS extension. There was no statistically significant increase in median survival in those patients receiving prophylactic whole-brain irradiation (median survival, 11.4 months) compared with the historical control patients of similar stage who did not receive prophylactic whole-brain irradiation (median survival, 10.5 months).

Toxicity

Leukopenia and thrombocytopenia were common and occurred equally in both groups. There were two hospital admissions for infection—one in each arm—and one toxic death. No complications of prophylactic whole-brain irradiation were recognized.

DISCUSSION

Radiation therapy frequently produces prompt regression in oat cell carcinoma (5). Following the demonstration that radiation therapy resulted in better survival than surgery (6), it became the standard initial approach. More recently, chemotherapy has been demonstrated to result in substantially increased survival time (7–10).

In this study employing POCC chemotherapy, irradiation with 3000 rads to involved sites not only failed to produce increased survival, but did not alter the pattern of relapse. If such involved-field radiation therapy were to be useful, it should at least achieve prevention or delay of local recurrence.

The relatively modest radiation dose employed in this trial has been utilized in many previous trials. Although it is efficient at inducing prompt regression of oat cell carcinoma, it now seems unlikely that it is capable of sterilizing residual tumor even in the face of an apparently satisfactory chemotherapy response. In addition, initial local relapses have occurred in patients receiving higher radiation doses. Five patients, who in an immediately prior study had received radiation to the thorax, relapsed initially at this site. Two of these patients had limited disease only and received 5000 rads to the chest, two received 4000 rads, and one received 3000 rads. The high incidence of local recurrence is undoubtedly a result of an improved survival following chemotherapy. Previously these patients died from metastatic disease so quickly that local recurrences were not seen. It may also be significant that many of the patients in the involved-field radiation arm relapsed soon after receiving their irradiation. Because of the time required for administration of radiation and the time required for recovery of the peripheral blood cell counts following radiation, there was a prolonged period (mean, 66 days) when no chemotherapy was given.

Oat cell cancer often extends to the brain (9,11). In consecutive Stanford studies 31 patients were treated with prophylactic whole-brain irradiation

with no cases of CNS extension. This contrasts dramatically with the preceding study (1) at this institution in which six cases of brain extension occurred in the 19 patients who did not receive prophylactic whole-brain irradiation. Survival was not affected by the use of whole-brain irradiation, presumably because current chemotherapy is not yet capable of providing long-term disease control in peripheral sites.

New trials in the management of oat cell cancer of the lung must focus on more effective chemotherapy. Radiation therapy to involved sites of disease may be further explored with higher doses of radiation therapy or after more effective initial control with chemotherapy although the optimal timing for such treatment is unclear. The demonstration of effective CNS prophylaxis by radiation suggests that the amount of residual tumor may be critical. We feel that the morbidity associated with CNS extension justifies prophylaxis despite our inability to demonstrate improved survival utilizing current chemotherapy programs.

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Carbenicillin, Cefazolin, and Amikacin as an Empiric Therapy for Febrile Granulocytopenic Cancer Patients [1,2]

J. Klastersky,* L. Debusscher, D. Weerts-Ruhl, and J. M. Prevost [3]

Infection is a major cause of morbidity and death in cancer patients, especially when severe neutropenia is present. In these patients, antimicrobial therapy is often undertaken before the recognition of the offending pathogen (1). Various antibiotic regimens to be used empirically in granulocytopenic febrile patients with cancer have been investigated and it appears that a combination of carbenicillin and an aminoglycoside would currently have the best ratio of efficacy to toxicity for initial empiric therapy under these conditions (2).

Previously, a combination of carbenicillin, cephalothin, and gentamicin had been found to be not more active but more toxic than a combination of carbenicillin and cephalothin (3). However, the efficacy of a triple combination of antibiotics including carbenicillin, cefazolin, and amikacin in febrile patients with cancer and neutropenia was investigated further. The latter regimen was expected to cover a wide spectrum of possible pathogens, including most gentamicin-resistant strains, and to take advantage of the antimicrobial synergism between penicillins or cephalosporins and amikacin. In addition, it was hoped that at the dosage used here, amikacin and cefazolin might be less nephrotoxic than gentamicin and cephalothin.

METHODS

The patients in this series were hospitalized at the Institut Jules Bordet which is the clinical center for cancer research at Brussels University. Fifty-two consecutive patients with disseminated cancer, neutropenia (< 1000 cells/mm^3), and fever (> 38.5°C) were admitted to the study, provided their serum creatinine level was < 1.5 mg/100 ml.

Therapy with iv administered carbenicillin disodium (10 g qid, over a period of 20 minutes), iv administered cefazolin (1 g qid, over a period of 20 minutes), and iv administered amikacin (500 mg bid, over a period of 20 minutes) was started as soon as a presumptive diagnosis of sepsis had been made, without waiting for the isolation and identification of the offending microorganism. As data became available, each febrile episode was classified as a microbiologically documented infection, a clinically documented infection, or a febrile episode without microbiologic or clinical documentation.

Before receiving the antibiotics, each patient had a complete history, physical examination, chest roentgenogram, blood cultures (three times over a 1-hour period), and cultures of possible foci. Other tests included: serum electrolytes, BUN, creatinine, glucose, and a cbc count including wbc and differential counts. In most patients these investigations were repeated during and after the treatment with carbenicillin, cefazolin, and amikacin.

Response to therapy was evaluated 72 hours after the start of antimicrobial treatment; no antibiotics other than those referred to above had been administered during this period of time. The treatment was considered to be a failure when no response or a minimal one to antibiotics was observed and the patient died primarily as a result of infection, or when therapeutic changes or additions were required. All patients who did not fail were considered successes. However, patients with documented bacterial infection who eventually survived but required a change of antibiotics after 72 hours of therapy were considered to have partially benefitted from the empiric regimen. When no infection could be identified and fever persisted despite complete re-evaluation and without positive findings from cultures, the antibiotics were discontinued after 72 hours and the patient was observed closely. The result of the antimicrobial therapy was then considered to be a failure.

The microorganisms studied in the present series were isolated and identified by standard methods. Their sensitivity to antibiotics was determined by

[1]Received Feb 28, 1977; revised June 20, 1977; accepted July 7, 1977.

[2]Carbenicillin: NSC-111071; CAS reg. No. 4800-94-6; malonamic acid, *N*-(2-carboxy-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]hept-6-yl)-2-phenyl-, disodium salt.

Cefazolin: CAS reg. No. 25953-19-9.

Amikacin: NSC-177001; CAS reg. No. 37517-28-5.

[3]Service de Médecine et Laboratoire d'Investigation Clinique, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, Belgium.

**Reprint requests to:* J. Klastersky, MD, Department of Medicine, Institut Jules Bordet, 1, rue Héger-Bordet, 1000 Brussels, Belgium.

the inocula-replicating method using Mueller-Hinton agar and inocula of 10^4–10^5 microorganisms per milliliter. Serum was obtained from the patients on the second day of treatment just before and 1 hour after the administration of the antibiotics. These sera were tested for bacteriostatic and bactericidal activity against the microorganisms that were considered to be responsible for the infection in each patient; suspensions of these bacteria, which were in a concentration of 10^4–10^5 microorganisms per milliliter in trypticase soy broth, were added to serial twofold dilutions of the sera. The highest dilution that failed to show evidence of macroscopic growth was considered to represent the bacteriostatic endpoint. Clear tubes were plated on blood-agar plates to determine the maximal bactericidal dilution.

RESULTS

A total of 52 patients (32 men and 20 women) were included in the present study over a 12-month period. Their mean age was 52.9 years (range, 16–78) and their mean weight was 63.3 kg (range, 54–71). The mean duration of therapy was 6.1 days (range, 3–11). As indicated in table 1, 26 of the 52 patients studied (50%) presented a microbiologically documented infection and 13 had a favorable response to the antibiotics. It should be pointed out that three episodes of infection were caused by fungi and that none of them, as could be expected, responded to the antimicrobial regimen. Clinically documented episodes, as defined earlier, were observed in 16 patients (31%) and no clinical source for a possible infection could be found in the ten remaining patients (19%) who were included in this series on the basis of a febrile episode. The respective favorable response rates to therapy were 81% and 60% in these groups of patients in whom no microbiologic documentation of the febrile episode could be obtained.

Twelve patients (23%) died; three of these died as a direct result of a bacterial infection. Another patient died as a result of a disseminated fungal infection on Day 3 after the start of antimicrobial therapy and before the pathogen was recognized and adequate antifungal therapy could be undertaken.

Table 2 analyzes various factors which might have influenced the outcome of the patients, excluding those who had a documented fungal infection before their death (three patients). The underlying neoplastic disease did not appear to influence the outcome significantly. The infections arising from foci in soft tissues responded favorably more often (75%) than pulmonary or urinary tract infections (59%); gram-negative septicemias originating from soft tissue infections also responded more often (83%) than septicemias arising from pulmonary or urinary sources (50%).

Patients with a serum creatinine level <1 mg/100 ml responded as often (66%) as those with a higher initial serum creatinine (63%). Among the patients whose initial granulocyte count was $> 100/mm^3$, six of eight patients (75%) responded whereas among the patients whose granulocyte count was $< 100/mm^3$ only 26 of 41 (63%) had a favorable response. The favorable clinical response was more frequent (73%) in the patients whose granulocyte count increased during therapy than in those whose level of granulocytes remained stable or decreased (56%).

The importance for the clinical outcome of the type of pathogen isolated from the treated patients is illustrated in table 3. As could be expected,

TABLE 1.—Overall response and death rate

	No. of patients (%)			
	Total	Favorable response	Died	Died from infection
Microbiologically documented episodes	26	13 (50)	6 (23)	3 (11)
Fungal infection	3	0	1	1
Gram-negative septicemia	14	9	4	1
Gram-negative infection	5	3	1	1
Gram-positive septicemia	4	1	0	0
Not microbiologically documented episodes	16	13 (81)	3 (18)	0 (0)
Clinically documented				
Pulmonary	9	7	2	0
Nonpulmonary	7	6	1	0
Fever only	10	6 (60)	3 (30)	1 (10)
Totals	52	32 (61)	12 (23)	4 (7)

TABLE 2.—Role of various factors possibly influencing the outcome

	No. of patients/No. of responses				
	Microbiologically documented episodes	Gram-negative septicemia	Clinically documented episodes	Fever only	Total
Total No.*	23/13	14/9	16/13	10/6	49/32
Underlying disease					
Lymphoma or chronic lymphocytic leukemia	5/2	4/2	4/4	0/0	9/6
Acute leukemia	9/6	4/3	5/4	5/3	19/13
Metastatic solid tumor	9/5	6/4	7/5	5/3	21/13
Site of infection					
Lungs	7/2	4/2	9/7	0/0	16/9
Urinary tract	5/3	4/2	1/1	0/0	6/4
Soft tissues	11/8	6/5	6/5	0/0	17/13
No site found	0/0	0/0	0/0	10/6	10/6
Level of serum creatinine (mg/100 ml)					
< 1.0	13/8	9/7	10/8	7/4	30/20
> 1.0	10/5	5/2	6/5	3/2	19/12
Granulocytopenia (cells/mm³)					
< 100	4/3	2/2	2/1	2/2	8/6
> 100	19/10	12/7	14/12	8/4	41/26
Increased during therapy	13/8	7/5	9/8	4/3	26/19
Remained stable during therapy	6/3	4/3	4/3	2/1	12/7
Decreased during therapy	4/2	3/1	3/2	4/2	11/6

*Excluding fungal infections.

TABLE 3.—Relationship between the nature of the pathogens, their sensitivity to the antibiotics, and the clinical outcome

	No. of patients/No. of responses		
	Total	Pulmonary infection (not septicemic)	Septicemias (septicemia associated with pulmonary infection)
Microorganisms			
Diplococcus pneumoniae	1/0		1(1)/0(0)
Staphylococcus aureus	3/1	0/0	3/1
Neisseria meningitidis	1/1	0/0	1(1)/1(1)
Salmonella	2/1	0/0	2/1
Escherichia coli	7/6	0/0	5/4
Klebsiella	4/1	1/0	3(2)/1(1)
Enterobacter	2/2	0/0	2/2
Pseudomonas aeruginosa	3/1	1/0	1(1)/0(0)
Candida albicans	2/0	1/0	1/0
Aspergillus	1/0	1/0	0/0
Sensitivity of pathogens (excluding fungi)			
Cefazolin, amikacin, and carbenicillin	12/8	0/0	9/7
Amikacin and carbenicillin	4/3	1/0	4/2
Cefazolin and amikacin	3/1	1/0	2/1
Amikacin	3/0	0/0	2/0
Cefazolin and carbenicillin*	1/0	0/0	1/0

*Pneumococcus.

TABLE 4.—Importance of the bactericidal activity of the serum for the clinical outcome in gram-negative septicemia

Bactericidal dilution of the serum	Clinical response	
	Failure	Favorable
1/8	1	1
1/16	1	0
1/32	0	0
1/64	2	2
1/128	1	4
1/256	0	1
≥ 1/512	0	1

fungal infections did not respond to the combination of antibiotics given. As far as microbial pathogens are concerned, there are too many different species involved in the infections studied here to allow a meaningful evaluation of the role of the nature of the pathogen for the outcome. The data stress, however, the poor prognosis of microbiologically documented infections arising from the pulmonary tract. Among seven patients with pulmonary infections caused by bacteria, only two responded favorably to the antimicrobial therapy.

Among the microbiologically documented infections 12 were caused by bacteria sensitive to all three antibiotics which were given and eight responded favorably (66%); on the other hand, only four patients of 11 (36%) whose infection was due to microorganisms resistant to one or two of the antibiotics used responded favorably. It is of special interest that none of the three infections caused by microorganisms sensitive to amikacin only responded.

The results of the determination of the serum bactericidal activity performed in the 14 patients in whom gram-negative septicemia could be documented are indicated in table 4. It can be seen that higher bactericidal dilutions of the serum were associated with a higher incidence of favorable clinical results. The geometric mean of the bactericidal activity was 1/64 in patients infected with an organism sensitive to all three antibiotics given

TABLE 5.—Summary of the clinical course of patients who failed to respond

Patient No./ age/sex	Underlying disease	Site of initial infection	Septicemia	Pathogen	In vitro sensitivity*		
					Cefazolin	Amikacin	Carbenicillin
1/41/M	Carcinoma, testis	Lungs	+	*Pneumococcus*	S	R	S
2/26/M	Lymphoma	Iv catheter	+	*Staphylococcus aureus*	R	S	R
3/31/M	Leukemia, acute myelogenous	Pharynx	+	*Staphylococcus aureus*	S	S	R
4/57/M	Carcinoma, lung	Lungs	0	*Klebsiella*	S	S	R
5/65/M	Leukemia, acute myelogenous	Lungs	0	*Pseudomonas aeruginosa*	R	S	S
6/70/M	Leukemia, chronic lymphocytic	Urinary tract	+	*Klebsiella*	R	S	R
7/49/F	Carcinoma, breast	Lungs	+	*Klebsiella*	S	S	S
8/53/M	Lymphoma	Gastrointestinal tract	+	*Salmonella*	S	S	S
9/24/M	Leukemia, acute myelogenous	Lungs	+	*Pseudomonas aeruginosa*	R	S	S
10/65/F	Carcinoma, breast	Urinary tract	+	*Escherichia coli*	R	S	R
11/53/M	Leukemia, acute myelogenous	Pharynx	0	—	—	—	—
12/59/F	Carcinoma, ovary	Lungs	0	—	—	—	—
13/60/F	Carcinoma, breast	Lungs	0	—	—	—	—
14/28/M	Leukemia, acute myelogenous	—	0	—	—	—	—
15/53/M	Carcinoma, parotid gland	—	0	—	—	—	—
16/53/M	Leukemia, acute myelogenous	—	0	—	—	—	—
17/54/F	Carcinoma, pancreas	—	0	—	—	—	—

*S = sensitive, R = resistant.
†Died as a result of infection.
‡Also received multiple transfusions of granulocytes after Day 3.

and 1/16 in those whose offending pathogen was sensitive to only one or two of the three antibiotics given.

Table 5 summarizes the courses of the 17 patients who were considered failures or partial responders to the antimicrobial triple therapy with carbenicillin, cefazolin, and amikacin, excluding patients with proven fungal infection. It can be seen that three patients died as a direct result of bacterial infection. One patient (No. 5) died as a result of infection caused by *Pseudomonas aeruginosa* in spite of therapy with carbenicillin and amikacin to which it was sensitive. Another patient (No. 10) died as a result of *Escherichia coli* (*E. coli*) infection originating from an infected and obstructed urinary tract; the pathogen was sensitive only to amikacin which was the only effective antibiotic given. A third patient (No. 14) with no clinical or microbiologic documentation of infection failed to respond to the triple combination; therapy was discontinued on Day 4 and the septicemia caused by *E. coli* could be documented; the patient died on Day 5. Fourteen other patients initially failed to respond to the combination of carbenicillin, cefazolin, and amikacin; in eight patients (Nos. 1–4, 6, 7, 11, and 16) the antimicrobial therapy was changed after Day 3 and these patients eventually survived. Six of these eight patients had a documented bacterial infection. In two patients (Nos. 8 and 9) death occurred in spite of a change in antimicrobial therapy. In one patient (No. 13) with a "clinically documented" lung infection, the final outcome was favorable in spite of poor initial response to antibiotics and no additional antimicrobial therapy; in that patient the existence of an infection can be doubted. Three other patients (Nos. 12, 15, and 17) died shortly after the discontinuation of the triple-antibiotic therapy.

Thus, besides the three patients described above who died from the initial infection, five others died during the administration of the antibiotics or immediately after their discontinuation. Although it

to initial therapy with carbenicillin, cefazolin, and amikacin

Initial granulocyte count (cells/mm³)	Granulocyte count during treatment	Clinical response	Blood cultures positive		Pathogen isolated	Other antibiotics given	Eventual outcome
			During therapy	At autopsy			
100	Increased	Poor	+	–	*Pneumococcus*	Penicillin	Favorable
100	Stable	Poor	+	–	*Staphylococcus aureus*	Vancomycin	Favorable
100	Increased	Poor	+	–	*Staphylococcus aureus*	Methicillin	Favorable
100	Increased	Poor	0	–	—	Cotrimoxazole	Favorable
100	Stable	None	0	+	*Pseudomonas aeruginosa*	—	Died on Day 5†
100	Increased	Poor	0	–	—	Cotrimoxazole	Favorable
100	Increased	Poor	+	–	*Klebsiella*	Cotrimoxazole	Favorable
100	Stable	Poor	0	–	—	Ampicillin	Died on Day 7
100	Decreased	Poor	0	–	—	Tobramycin	Died on Day 10‡
100	Increased	Poor	0	+	*Escherichia coli*	—	Died on Day 7†
100	Stable	None	0	–	—	Amphotericin	Favorable
100	Decreased	None	0	+	*Candida*	—	Died on Day 5
100	Increased	Poor	0	–	—	—	Favorable
100	Decreased	None	0	+	*Escherichia coli*	—	Died on Day 5†
100	Stable	None	0	–	—	—	Died on Day 4
100	Decreased	None	+	–	*Enterobacter*	Cefazolin + amikacin	Favorable
100	Increased	Poor	0	–	—	—	Died on Day 8

is difficult to exclude the role the initial infection (whether presumed or documented) had in the fatal outcome, these patients were considered to have died from other causes, namely, extensive and unresponsive neoplastic disease.

Serious side effects which could possibly be related to the administration of the triple-antibiotic therapy were few in this study. A rise in the serum creatinine level above the upper normal value (> 1.5 mg/100 ml) was seen during therapy in two patients (3.8%); in one patient the serum creatinine level rose from 0.7 to 2.2 mg/100 ml and in the other patient it rose from 1.1 to 2.9 mg/100 ml. In both patients, the serum creatinine level returned to normal within a few days after the discontinuation of the antibiotics. Two other patients had a transient elevation of the BUN during therapy without a concomitant rise in the serum creatinine level, and these changes might therefore have been unrelated to the administration of the antibiotics.

Hypokalemia (potassium serum level < 3.0 mEq/liter) was observed in 24 of 30 patients (80%) who had a normal pretreatment serum potassium level. The mean level of serum potassium in these 30 patients was 3.7 mEq/liter before therapy with the triple-drug combination and 2.6 mEq/liter (range, 1.6–3.1) during therapy. Forty-two of 52 patients in this series (80.7%) received potassium salts either as a prophylaxis or as a treatment for antibiotic-induced hypokalemia. No toxic effects which could have been related to the hypokalemia were observed in this series.

Among other toxic reactions, phlebitis at the site of the iv injections developed in 13 of the patients (25%). Allergic cutaneous rashes were observed in two patients (3.8%) during therapy. In two patients (3.8%) profuse hemorrhage developed during therapy with antibiotics; in one patient epistaxis occurred and in the other bleeding originated from a postoperative wound. Finally, one patient (1.9%) developed vertigo and dizziness during the administration of the triple-drug combination; this patient had received large amounts of gentamicin during the weeks preceding the present treatment. Cochlear function remained unaltered and the abnormalities of the vestibular function disappeared within a few days after discontinuation of therapy with carbenicillin, cefazolin, and amikacin.

DISCUSSION

Therapy with multiple-drug combinations without knowledge of the offending pathogen of presumed sepsis is not usually recommended as a sound procedure in the therapy of infectious diseases. However, it is likely that early empiric antimicrobial therapy is beneficial in patients with severely impaired mechanisms of defense against infection, especially in neutropenic patients (1). On the other hand, in many modern hospitals a substantial proportion of pathogenic microorganisms are resistant to several common antibiotics, sometimes including gentamicin. These are the reasons why we have investigated the efficacy in neutropenic cancer patients of a combination of carbenicillin, cefazolin, and amikacin. Earlier, in this laboratory, a similar combination of carbenicillin, cephalothin, and gentamicin had been found to be equally active but more nephrotoxic than carbenicillin plus cephalothin (3).

Our rate of favorable responses in microbiologically documented bacterial infections is similar to that recently found in the same type of patients by a large cooperative group of investigators who compared the combinations of carbenicillin plus cephalothin, carbenicillin plus gentamicin, and gentamicin plus cephalothin (2). In that study, however, 8.5% of the patients had infections which were caused by a microorganism resistant to both antibiotics used and 89% of these patients failed to respond.

In our study, 13 of 26 patients with documented bacterial infections responded favorably to the triple-drug therapy; in addition, the six patients with microbiologically documented bacterial infections who failed to respond optimally to the triple-drug combination survived after having required a change in the antimicrobial regimen. Thus, the use of the combination of carbenicillin, cefazolin, and amikacin was eventually associated with an overall survival of 19 of 26 patients (73%) with bacterial infection.

On the other hand, our study indicates a low rate of renal function impairment (3.8%) which contrasts with the higher frequency of nephrotoxicity associated with the use of the combination of carbenicillin, cephalothin, and gentamicin (17%) or cephalothin plus gentamicin (12%) reported more recently (2,3). In these studies, and in some others in which nephrotoxicity has been linked to the combined use of cephalothin and gentamicin or cephalothin and tobramycin, high doses of cephalothin have been used (4). This suggests the possibility that the occurrence of nephrotoxicity associated with these combinations might have been related to the use of a high dosage of cephalosporins.

The present study, limited to a small number of patients, served as a preliminary trial of the triple-drug combination for a cooperative study designed to compare carbenicillin plus amikacin and the triple-drug regimen tested here (5). The small num-

ber of patients studied makes difficult the analysis of various factors which may have an important role for the outcome of severe infections in granulocytopenic cancer patients. These factors have recently been analyzed in a large clinical study (2). Our investigation confirms that the response of patients depends primarily on whether or not the granulocyte count will increase during therapy; 73% of the patients improved when the peripheral granulocyte count rose compared to only 56% who improved when the granulocyte count did not rise. Transfusions of granulocytes were not used in the present series as an adjunct to antimicrobial therapy; the granulocyte transfusions might have changed the outcome in the three patients who died from bacterial infection, since some clinical trials attest to the value of granulocyte transfusions for the granulocytopenic patient with progressive infection (6). Transfusions of granulocytes thus appear to be required in a small minority of infected patients and further studies should delineate the factors which are predictive of a possible benefit of these transfusions to the patients.

Since effective therapy is now available for some malignant diseases, successful management of infections in patients with cancer becomes increasingly important. The present study indicates that the combination of carbenicillin, cefazolin, and amikacin, administered as indicated in this paper, is a highly effective antimicrobial therapy in febrile granulocytopenic patients with cancer. It provides a broad antimicrobial coverage and it is associated with a low frequency of renal dysfunction.

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Chemotherapy for Adult Acute Nonlymphocytic Leukemia With Daunorubicin and Cytosine Arabinoside [1,2]

Peter A. Cassileth* and Martin E. Katz [3,4]

SUMMARY

Fourteen of 21 adult patients (67%) with acute nonlymphocytic leukemia achieved a complete remission (CR) after receiving combination chemotherapy with daunorubicin and cytosine arabinoside (ara-C). Eight of the 14 CRs developed after a single course and four of 14 after two courses of induction therapy making initial hospitalization relatively brief (median, 38 days). Four of five patients > 60 years old achieved CR. The induction therapy was repeated monthly up to the dosage limits imposed by daunorubicin cardiotoxicity in an attempt to lengthen subsequent remission duration. The median duration of CR was 12 months which compares favorably with previously reported series. In this series, treatment with 3 days of daunorubicin and 7 days of ara-C proved to be a highly effective induction regimen for patients with acute nonlymphocytic leukemia regardless of age. The improved duration of CR may be a manifestation of the extent of initial leukemic cell-kill in successful induction and consolidation therapy rather than an effect of maintenance therapy cycles.

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The median survival of adults with acute nonlymphocytic leukemia improved but little from the 1950s to the 1960s (1). The utilization of daunorubicin in therapeutic regimens has increased the incidence of complete remissions (CRs) in untreated patients to 49%–55% (2,3) when used alone and to 53%–82% when used in combination with other agents, principally, cytosine arabinoside (ara-C) (4–9). With CR rates of > 50%, the median survival of patients has improved and greater attention has been directed to the problem of enhancing remission duration. The study below explored the potential advantages of intensive consolidation therapy by continuing the induction regimen of daunorubicin and ara-C immediately after CR until the cardiotoxic dose-limiting level (500–550 mg/m²) of daunorubicin had been reached in conjunction with prophylactic intrathecal methotrexate.

PATIENTS AND METHODS

Patients

All adult patients with acute nonlymphocytic leukemia not previously treated with daunorubicin or adriamycin who entered the hospital of the University of Pennsylvania between June 1975 and November 1976 were eligible for the protocol. Patients with blast cell transformation of chronic myelogenous leukemia or smoldering acute leukemia were excluded. Twenty-four eligible patients were admitted to the hospital during this time. One man (age, 64 years) with chronic obstructive pulmonary disease, cirrhosis of the liver, congestive heart failure, and renal insufficiency, and one woman (age, 70 years) with severe organic mental deterioration, were not treated for their disease. A third patient (age, 39 years) had the simultaneous presentation of a massive mediastinal teratoma and was not

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[3]Hematology-Oncology Section, Hospital of the University of Pennsylvania, Philadelphia.

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**Reprint requests to:* Peter A. Cassileth, MD, Hospital of the University of Pennsylvania, Maloney 578, 3400 Spruce St, Philadelphia, Pa 19104.

included. Of the 21 patients entered, two had received a single course of COAP (cyclophosphamide, vincristine, ara-C, and prednisone) without improvement before referral to the hospital. The rest of the patients had not been treated previously. The 21 treated patients (12 women and nine men), aged 17–67 years (median, 42 years), are all included in the analysis of the results. Eight patients were 17–36 years old, eight patients were 41–57 years old, and five patients were > 60 years old (range, 60–67 years). A 17-year-old man had acute promyelocytic leukemia; one man (age, 28 years) and one woman (age, 36 years) had acute undifferentiated leukemia; the remainder had acute myelocytic or myelomonocytic leukemia.

Initial Evaluation and Therapy

All patients were HLA typed, and routine blood cell counts, liver and kidney function tests, coagulation studies, anergy skin tests, chest roentgenogram and electrocardiogram, and test for hepatitis B_s antigen (Hb_sAg) were obtained. Routine blood cell counts, blood chemistries, chest roentgenogram, and electrocardiogram were obtained before each subsequent course. All patients had a lumbar puncture before therapy and spinal fluid tested for cells, protein, and sugar. The spinal fluid was also examined by the cytocentrifuge-generated smear technique even if the cell count was zero.

If fever was present, appropriate cultures and diagnostic tests were obtained and antibiotics were begun where indicated. Thrombocytopenia and anemia were corrected with transfusions. When the peripheral leukemic blast cell count was > 75,000/mm^3, 1.5 g of hydroxyurea was given orally every 6 hours until the blast cell count was reduced below this level. Allopurinol was administered at a dose of 300 mg/day orally before initial induction therapy and was used in subsequent courses only if the pretreatment uric acid was > 7 mg/dl.

Approval of the protocol by the Human Studies Committee of the University of Pennsylvania was secured, and informed consent was obtained from each patient entered into the study.

Chemotherapy Protocol

The induction phase of therapy consisted of daunorubicin, 45 mg/m^2/day on Days 1, 2, and 3 by iv push, and ara-C, 100 mg/m^2/day on Days 1–7 by continuous iv drip. Bone marrow examination was performed shortly after the nadir of this first course. If CR was not obtained after the first course, one or two additional cycles of the same drugs were given 2–4 weeks apart in the following doses: daunorubicin, 45 mg/m^2/day on Days 1 and 2 by iv push, and ara-C, 100 mg/m^2/day on Days 1–5 by continuous iv drip.

Failure to achieve a CR after the third course excluded such patients from the balance of the protocol. Patients achieving a CR were given monthly cycles of 2 days of daunorubicin and 5 days of ara-C as above plus intrathecal methotrexate (10 mg/m^2, to 15 mg maximum dose) on the third day of each cycle with oral citrovorum factor (6 mg every 6 hours × 8 doses) until a total dose of 500–550 mg/m^2 of daunorubicin was reached. Those patients remaining in CR received maintenance therapy commencing 1 month later consisting of BCNU, 200 mg/m^2 iv on Day 1, and cyclophosphamide, 1 g/m^2 iv on Day 1, on Months 1, 4, 7, 10, 13, 16, 19, and 22. On months 3, 6, 9, 12, 15, 18, and 21 ara-C, 50 mg/m^2 sc every 12 hours × ten doses, cyclophosphamide, 1 g/m^2 iv on Day 1, and vincristine, 2 mg iv on Day 5 were given with intrathecal methotrexate and systemic citrovorum factor (as above under consolidation therapy).

Nadir blood cell counts were obtained after each cycle and the dosage of drugs was adjusted downward with subsequent cycles if septic complications occurred.

Supportive Therapy

All patients were treated without isolation procedures or oral non-absorbable antibiotics. Presumptive infections were treated with cephalosporin, gentamicin, and carbenicillin pending culture documentation of the etiology. Granulocyte transfusions were employed in six febrile neutropenic patients as part of a randomized granulocyte transfusion study (10). Platelet transfusions were used when the platelet count was < 10,000/mm^3 or if clinically significant bleeding occurred with a higher platelet count. Random donor platelets were used initially; HLA-matched platelets were used for thrombocytopenia refractory to random donor platelets. Packed rbc transfusions were used as well when clinically indicated.

RESULTS

Remission Induction

Fourteen of the 21 patients (67%) achieved a CR. Eight of the 14 CRs developed after the first course of therapy, four after two cycles, and two after three cycles. Of the six patients requiring more than one drug cycle to gain CR, all had substantial partial remissions (PRs) after the initial course. CRs were obtained in seven of eight patients (88%)

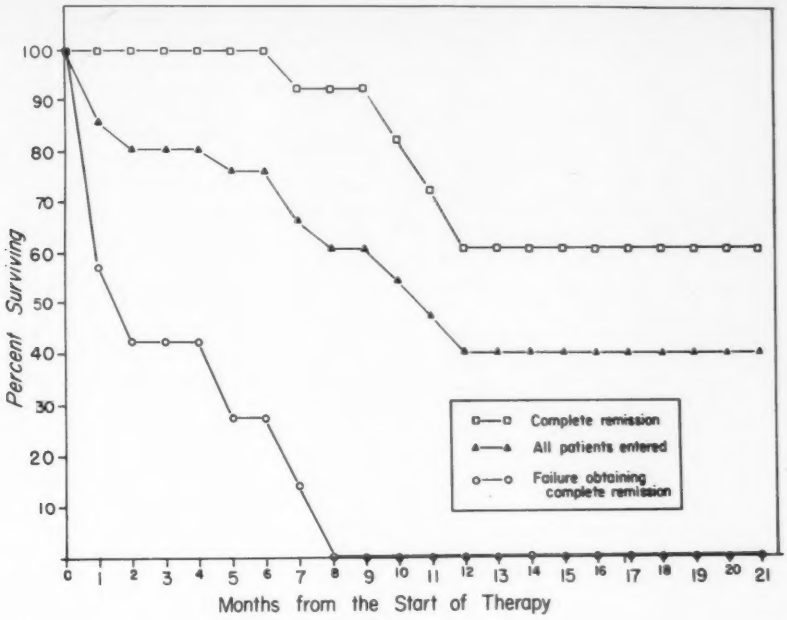

FIGURE 1.—Actuarial survival of patients with acute nonlymphocytic leukemia.

aged 17–36 years, three of eight (38%) aged 41–57 years, and four of five (80%) aged 60–67 years. The time from treatment initiation to CR ranged from 25 to 99 days (median, 38 days). Of the 14 patients entering the consolidation phase of therapy, five relapsed before completing consolidation and seven were begun on maintenance chemotherapy 4–10 months (median, 5 months) after obtaining CR. Two patients have not yet completed consolidation therapy because of complicating hepatitis in one case and persisting fungal infection in the other.

Remission Duration and Survival

The median duration of CR by actuarial analysis is 12 months, and eight patients have relapsed at 2, 3, 3, 5, 8, 8, 12, and 15 months; six patients remain in remission, three for remission durations of 13, 17, and 18 months. Of the eight relapsed patients, four died 3, 4, 4, and 5 months after relapse and four patients are alive 1, 2, 5, and 5 months after relapse. No patient who relapsed has gained a second CR with other therapy (including five patients treated with 5-azacytidine).

Survival data from the initiation of protocol therapy for all patients are shown in figure 1. The median survival of the 21 patients is 10.5 months. The eight patients not achieving a CR had a median survival of 1.5 months. Of these eight patients, three presented initially with pneumonia of uncertain etiology and were begun on chemotherapy before resolution of their pneumonia was established. They died in < 1 month. Of patients achieving a CR, the median survival is > 12 months. These results are summarized in table 1.

Toxicity

No patient died of hemorrhagic complications. Cardiotoxicity did not occur in any patient and

TABLE 1.—Summary of results of treatment of adult nonlymphocytic leukemia

	Patients obtaining CR	Patients failing induction therapy
Total No. of patients	14	7
Age range in yrs (median)	17–67 (38)	35–67 (55)
Median survival from start of therapy (mos)	12+	1.5
Median duration of remission (mos)	12	—

electrocardiograms obtained before each therapy course showed no alterations. Bacterial infections were common in the severely agranulocytic phase of therapy. Three patients developed systemic candidiasis with *Candida tropicalis* which persisted after CR was obtained. One infection resolved with miconazole therapy (11), one patient died in relapse 3 months after a 3-month remission with persistent candidemia despite amphotericin therapy, and in one patient a visceral *Candida* infection slowly resolved after extensive antifungal therapy.

Although one third of the patients developed elevations of serum-conjugated bilirubin levels after the initial course of induction therapy, hepatic enzymes were not abnormal, and subsequent readministration of the induction regimen did not induce liver function abnormalities (12). Two months after CR, one patient developed mild liver function abnormalities (Hb_sAg negative) which became normal 2 months later. Repeated therapy on two occasions with the induction regimen as part of consolidation therapy produced evidence of hepatic dysfunction. She has not been re-treated in the past 4 months pending return of liver function tests to normal. She feels well and remains in CR. In contrast, one patient developed typical signs and symptoms of infectious hepatitis (Hb_sAg negative) 2 weeks after CR; these signs and symptoms resolved rapidly in 2 weeks. Re-treatment caused no recrudescence of liver function abnormalities.

Two patients, a 17-year-old man and a 26-year-old woman, developed acute appendicitis early in remission and responded well after appendectomy. Aside from these occurrences, patients who achieved CR tolerated subsequent consolidation and maintenance therapy well. Of all treated patients, only one of the consolidation courses and four of the maintenance courses led to septic complications requiring hospitalization. These four episodes occurred 7–10 days after the cyclophosphamide, ara-C, and vincristine regimen requiring dosage adjustments in subsequent courses.

Central Nervous System Leukemia

One patient presented with meningeal leukemia at the outset of therapy. After five doses of intrathecal methotrexate, spinal fluid leukemic blast cells disappeared and did not recur. She has remained in CR on this protocol for the past 18 months. In this study, patients had serial lumbar punctures for prophylactic methotrexate as specified in the treatment protocol, and spinal fluid was examined for leukemic cells on each occasion by cytocentrifuge smear. No patient relapsed with central nervous system leukemia as the first manifestation of relapse. In fact, only one patient has subsequently developed meningeal leukemia and this occurred at a time of florid bone marrow relapse with a peripheral blast cell count of 100,000/mm^3.

DISCUSSION

The pilot study of Yates et al (6) yielded a 62% CR rate in patients treated with 3 days of daunorubicin and 7 days of ara-C. Similar results have been obtained when these two drugs were used alone for the same course durations (7), when adriamycin was substituted for daunorubicin (13), or when other drugs were added (14). One study in which 7 days of 6-thioguanine were added to the regimen reported an 82% CR rate (4). When fewer days of therapy with daunorubicin (15), or ara-C (8,16), or both (2,7,17) were used, the CR rate was lower. The CR rate of the present study (67%) in a larger group of patients confirms the efficacy of the initial study (6). The fact that one induction therapy course was adequate to yield a CR for eight of 14 patients allowed a relatively short initial hospitalization (median time of therapy to CR, 38 days).

It has been stated that older patients fare poorly with therapy (18). On occasion, therapeutic trials have excluded patients > 60 years old (7). However, four of five of our patients (80%) ≥ 60 years old achieved CR, and their remission lengths were as durable as those of younger patients, as has been noted by others (4,19).

Because of the estimated persistence of 1 billion leukemic cells in patients in CR (20), a variety of chemotherapeutic regimens have been employed to reduce the residual leukemic population and improve the duration of CR. In this study, we repeated at monthly intervals the successful induction regimen to see if early intensive consolidation with daunorubicin (to the limits imposed by its cardiotoxicity) would be beneficial. The median duration of CR in this study (12 months) compared favorably with median CR durations of 5–11 months using daunorubicin in combination with other drugs for induction therapy (2,4,5,14,17).

A study using a non-daunorubicin-containing induction regimen (21) revealed that the length of unmaintained CR was approximately 4 months. Studies where the drugs of the induction regimen were continued as consolidation and maintenance therapy yielded the following median CR durations: for ara-C and 6-thioguanine, 4 months (22); for POMP (prednisone, vincristine, methotrexate, and 6-mercaptopurine), 6 months (23); for CAM (cyclophosphamide, ara-C, and methotrexate), 7 months (24); and for COAP (cyclophosphamide, vincristine, ara-C, and prednisone), 10.5 months (25).

A variety of maintenance therapy programs with drugs other than those used in the remission induction regimen have been employed without significant improvement of the CR duration. One exception was the use of single doses of BCNU and cyclophosphamide every 2 months after initial induction therapy (and three consolidation courses) with cyclophosphamide, ara-C, and vincristine, yielding a median CR duration of 20.5 months (26). It is difficult to be sure in the present study or in the above-cited studies whether the initial, consolidation, or maintenance therapy is responsible for the gradually improving CR duration.

Although the value of maintenance therapy programs in childhood acute lymphocytic leukemia is well demonstrated (27), it remains to be shown that present maintenance therapy approaches in adults with acute nonlymphocytic leukemia enhance CR duration. It may be that the remission-inducing regimen has such a variable capacity to reduce the leukemic population initially that the re-emergence of clinically evident leukemia is variably delayed. The 1-year median duration of CR in the present study may then reflect only the efficacy of the initial daunorubicin-containing induction and consolidation therapy. That this may be the case is reflected by the findings of a 7-month (minimum) to 15-month (estimated) median CR duration in patients treated with daunorubicin and ara-C who were not treated after CR was established (16).

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Treatment of Metastatic Germ-Cell Tumors in Men With Adriamycin, Vincristine, and Bleomycin [1,2,3]

Michael A. Burgess,[4,*] Lawrence H. Einhorn,[5] and Jeffrey A. Gottlieb [†,4]

SUMMARY

Twenty-five patients with metastatic germ-cell tumors were treated with a combination of adriamycin, vincristine, and bleomycin. Fifteen patients (60%) had previously received radiation therapy, chemotherapy, or both. Twenty patients (80%) responded to treatment, with eight (32%) patients achieving a complete remission (CR) and 12 (48%) patients achieving a partial remission (PR). The median duration of response for the patients with PR was 4 months, whereas four patients with a CR remain alive without evidence of disease for > 3 years after the initiation of treatment. Responses were observed in all tumor categories and apparently were not influenced by prior therapy. Side effects included gastrointestinal toxicity, alopecia, neuropathy, skin changes, mucositis, and myelosuppression (more severe in previously treated patients). Though moderate success was demonstrated for this chemotherapy regimen, it does not appear as effective as more recent regimens including vinblastine and bleomycin.

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A variety of chemotherapeutic agents, when used alone, are capable of inducing objective tumor regression in male patients with metastatic nonseminomatous germ-cell tumors (1,2). These include actinomycin D (3,4), mithramycin (5,6), methotrexate (7), vinblastine (8), and the alkylating agents (9,10), in addition to the newer agents adriamycin (11), bleomycin (12), and *cis*-dichlorodiammineplatinum(II) (13). Unfortunately, most of these single-agent responses have been less than complete and usually of short duration. More recently, combination chemotherapeutic regimens utilizing vinblastine and bleomycin have significantly improved both the complete response (CR) rate and subsequent survival in this patient population (14–18).

In 1972, many of the patients with metastatic testicular cancer referred to the Department of Developmental Therapeutics at the M. D. Anderson Hospital had received prior radiation therapy and chemotherapy, usually with actinomycin D. A combination chemotherapy regimen was thus initiated utilizing agents to which these patients had not previously been exposed, namely, adriamycin, vincristine, and bleomycin, each of which had shown preliminary activity in patients with metastatic testicular cancer (12,19–22).

PATIENTS AND METHODS

Male patients with documented metastatic germ-cell tumors were considered eligible for the study. Patients who had previously received and had proven refractory to any of the study drugs were excluded. All previously untreated patients received adriamycin, 75 mg/m² iv every 21 days, and vincristine, 1 mg iv followed 6 hours later by bleomycin,

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[4]Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[5]Department of Medicine, Indiana University Medical Center, Indianapolis.

[†]Deceased.

[*]*Reprint requests to:* Michael A. Burgess, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

15 units im or iv. Vincristine and bleomycin were given twice a week to the first 11 patients. The development of excessive stomatitis and neuropathy in these patients necessitated a change in the schedule; the remaining 14 patients received vincristine and bleomycin in dosages of 1 mg and 30 units, respectively, given 6 hours apart, once weekly. The adriamycin dosage was reduced 20% in four patients who had received prior radiation and chemotherapy. Vincristine and bleomycin were delayed in patients if stomatitis existed when the dosages were scheduled to be given, and vincristine was deleted in patients who developed severe neuropathy. The total dosage of adriamycin was limited to between 450 and 550 mg/m^2 and the dose of bleomycin to 360 units. For patients reaching this upper dosage limit of adriamycin, maintenance chemotherapy consisted of either vinblastine, 0.1–0.2 mg/kg/day iv for 2 consecutive days each month, or actinomycin D, 2.5–4.0 mg iv as daily doses (0.5–1.0 mg) or as a single injection each month, depending on the patients' prior chemotherapy exposure. Maintenance chemotherapy was continued until there was evidence of progressive disease or until the patient had received chemotherapy for 2 years.

Serum and urinary levels of human chorionic gonadotropic (HCG) hormone were determined in most patients before and during therapy. During the time of the study, the more specific β-HCG determination (23) was not available. α-Fetoprotein determinations were not performed routinely on all patients, so that insufficient values were available for analysis.

A CR is defined as the disappearance of all evidence of metastatic disease for a minimum period of 1 month. Partial response (PR) is defined as a $>50\%$ reduction in the product of the diameters of measured lesions, provided there was no increase in the size of any lesion nor the appearance of new lesions. The duration of CR and PR was determined from the time that response was achieved until progressive disease became evident. Progressive disease is defined as a $>25\%$ increase in the size of measured lesions or the appearance of new lesions.

RESULTS

Between October 1972 and June 1974, 25 male patients with metastatic germ-cell tumors were treated, 16 at the Department of Developmental Therapeutics at the M. D. Anderson Hospital and nine at the Indiana University Medical Center; seven of these patients have been reported (24). The clinical characteristics of the patient population are shown in table 1. The median age of the group was 24 years and the median duration from the diagnosis to the start of protocol treatment was 4 months. Twenty-two of the patients had clinical stage III disease and three patients had stage II disease. The diagnosis of primary testicular cancer had been established by orchiectomy in 20 patients; in 11 patients a retroperitoneal lymph node dissection was subsequently performed. Fifteen patients had received prior radiation or chemotherapy; of these, 11 had received both treatment modalities prior to entry in the study. Of the 14 patients with prior chemotherapy exposure, 11 had received actinomycin D, either alone or in combination (table 2).

TABLE 1.—Clinical characteristics

Characteristic	No. of patients
Total	25
Age (yrs)	
Median, 24	
Range, 17–39	
Duration of disease prior to study treatment (mos)	
Median, 4	
Range, 1–36	
Clinical stage	
II	3
III	22
Prior treatment	
Surgery	
Orchiectomy	20
Retroperitoneal node dissection	11
Radiation	12
Chemotherapy	14

The response to treatment as it relates to the tumor cell type (Dixon and Moore classification) (25) is shown in table 3. Twenty (80%) patients responded to the chemotherapy regimen; eight (32%) achieved a CR and 12 (48%) achieved a PR. Five patients failed to respond to a minimum of three courses of treatment, as evidenced by progression of their metastatic disease. Patient responses were observed in each pathologic category. The patient with pure seminoma achieved a PR of his widely metastatic pulmonary, nodal, and bone disease which lasted 5 months. Of the 12 patients in pathologic class II with embryonal carcinoma (including two patients with seminomatous elements), three CRs and six PRs were observed. All three patients achieving a CR had received prior chemotherapy and, of these, two had received radiation therapy. Three of the six patients who achieved a PR had received prior radiation therapy and chemotherapy. Of the seven patients in classes III and IV, three achieved a CR; two of these patients remain free of any evidence of recurrent disease 34 and 45 months from the onset of CR. The third patient died of radiation pericarditis 1 month after

TABLE 2.—Response related to prior chemotherapy

Prior therapy	Total No. of patients	Response to protocol treatment (No. of patients)		
		CR	PR	Progressive disease
Actinomycin D	5	2	1	2
Mithramycin	2	1	1	
Actinomycin D + chlorambucil	2		1	1
Actinomycin D + cyclophosphamide + methotrexate	1		1	
Actinomycin D + chlorambucil + methotrexate	1		1	
Actinomycin D + bleomycin	1		1	
Actinomycin D + mithramycin	1	1		
Vinblastine	1	1		
Totals	14	5	6	3
No prior chemotherapy	11	3	6	2
Totals	25	8	12	5

achieving a CR. No evidence of tumor was found at autopsy.

Four patients in class V had pathologic evidence of choriocarcinoma elements in their tumors, and two of these patients had pure choriocarcinoma. All four patients showed some response to the chemotherapy with two CRs and two PRs. The patients who achieved a CR both had stage II nodal disease and had not received prior therapy. These two young men remain free of recurrent disease 38 and 47 months from the onset of CR. Both patients who achieved a PR had received prior chemotherapy, and the durations of response were 2 and 5 months. The patient with yolk-sac endodermal carcinoma achieved a PR for 7 months.

Table 2 shows the response as it relates to prior chemotherapy exposure. Of the 11 patients who had received no previous chemotherapy, nine (82%) responded with three CRs and six PRs. Of the 14 patients who had relapsed while receiving previous chemotherapy (including actinomycin D in 11 patients), 11 (78%) responded with five CRs and six PRs.

Objective evidence of tumor regression was evident in all patients who subsequently manifested either a CR or a PR after one course of therapy. In many responding patients a PR status was already achieved at the time a second course was given.

HCG determinations were obtained in 22 cases; for nine the level of HCG was elevated in either the serum or urine or both. Serum levels were highest in two patients with choriocarcinoma at 8000 and 850,000 units, respectively. The former patient achieved a PR with a fall of the serum HCG to 200 units, whereas the latter patient had a CR associated with a fall of the serum HCG to undetectable levels following four courses of chemotherapy. In other patients, fluctuations in the levels of HCG usually coincided with evidence of progressive disease or response to treatment.

At the start of treatment, pulmonary parenchymal nodules were evident in 18 patients and were the only manifestation of stage III disease in eight patients. Other sites of metastatic disease included, in decreasing frequency, lymph nodes (abdominal, mediastinal, and supraclavicular), liver, bone, and brain. Pulmonary metastases were the most responsive to chemotherapy, with four CRs in the eight patients who had this as their sole manifestation of metastatic disease.

The median duration of response for those 12 patients achieving a PR was only 4 months with a range from 2 to 12 months. However, for the eight patients achieving a CR, the durations of response were 2, 5, 7, 22, 34+, 38+, 45+, and 47+ months. Four patients have had no evidence of disease for >1 year after cessation of chemotherapy. Two of these patients were stage II at the onset of treatment and neither had received prior therapy.

Cerebral or cerebellar metastases developed in five patients who had achieved a PR of other visceral disease. Concomitant evidence of visceral relapse was present in four of these patients.

The toxicity related to this chemotherapy regimen

TABLE 3.—Response related to tumor cell type

Pathologic class (Dixon and Moore)	Total No. of patients	Response (No. of patients)		
		CR	PR	Progressive disease
I	1		1	
II	12	3	6	3
III	3	1	1	1
IV	4	2	1	1
V	4	2	2	
Other*	1		1	
Totals	25	8	12	5

*Yolk-sac endodermal carcinoma.

was variable, though all patients sustained alopecia and mild upper gastrointestinal symptoms due to the adriamycin. When the vincristine and bleomycin were given initially twice a week, excessive stomatitis was observed, a consequence of both the adriamycin and bleomycin. Neuropathy was dose-limiting as a result of the vincristine, and bleomycin-related skin changes were observed in a small number of patients. When the schedule was changed to weekly administration of the vincristine and bleomycin, tolerance was significantly improved without changing the total weekly dosage of bleomycin. One patient developed pulmonary symptoms and an interstitial infiltrate, which might possibly have been related to bleomycin pulmonary toxicity. Another patient developed a prolonged systolic time interval after 525 mg/m² of adriamycin without clinical evidence of myocardial damage.

Myelosuppressive toxicity was limited almost exclusively to granulocytopenia except in those patients who had received prior radiation therapy or chemotherapy. None of the previously untreated patients required a dosage reduction of the adriamycin for reasons of myelosuppression. In these patients, the median lowest white blood cell and granulocyte counts were 2.3×10^3/mm³ and 1.2×10^3/mm³, respectively, and no patient had thrombocytopenia of $< 100{,}000$ cells/mm³. When the bone marrow reserve had been impaired by previous radiation therapy or chemotherapy, the myelosuppressive toxicity was excessive, and 11 patients had an initial or subsequent reduction in the dosage of adriamycin to 45–60 mg/m². Four of these patients required hospitalization with fever and granulocytopenia (< 500 cells/mm³), yet no drug-related mortality or thrombocytopenic hemorrhage was observed.

DISCUSSION

The combination of adriamycin, vincristine, and bleomycin was chosen as therapy for patients with metastatic testicular carcinoma since, at the time of initiation of the study, most patients had previously been exposed to the then more conventional drugs such as actinomycin D, mithramycin, vinblastine, and the alkylating agents. Vincristine and bleomycin were given 6 hours apart because of in vitro evidence of improved effectiveness of the bleomycin following synchronization of cells with vincristine (26).

The overall objective response rate of 80% in this study compares favorably with other combination drug regimens for these tumors (1,15–18). The response rate seemed independent of whether the patients had previously received chemotherapy. Objective tumor regression, including CR, was observed in all pathologic tumor types. However, as in other reported series, the duration of response for those 11 patients achieving a PR was brief; the median duration was 4 months. Nevertheless, eight (32%) patients achieved a CR, with four (16%) remaining free of disease for >3 years from the onset of treatment. Using a similar drug regimen, Seeber et al (27) obtained a 50% response rate in 16 evaluable patients, with five (31%) patients achieving a CR. Four of these patients had remained in remission between 2 and 11 months at the time of the report. Kardinal et al (28) reported a 22% response rate in 27 evaluable patients; three (11%) patients had a CR. In this study using adriamycin, bleomycin, and vinblastine, the lower response rate may reflect a different (lower) dosage schedule for both the adriamycin and bleomycin.

Despite the encouraging results reported herein,

CR rates of >40% are now being achieved with vinblastine and continuous-infusion bleomycin combinations (29), which appear to be even further improved by the addition of *cis*-dichlorodiammineplatinum(II) (30,31). Furthermore, CRs are being observed with these regimens in patients with massive metastatic disease, a result not seen in the present series.

Further studies employing multiple active drugs for the induction and maintenance of remission continue. Clearly, the prognosis for the patient with stage III metastatic germ-cell malignancy is steadily improving, and with the judicious use of aggressive adjuvant chemotherapy regimens for patients with stage II disease, more long-term survivors can be anticipated.

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Methyl-CCNU Versus Methyl-CCNU and 5-Fluorouracil in Carcinoma of the Large Bowel [1,2,3]

Lynn E. Posey and Lee Roy Morgan [4,5,*]

SUMMARY

5-Fluorouracil (5-FU) and methyl-CCNU have demonstrated separate sensitivities in carcinoma of the large bowel. This study was an attempt to see if methyl-CCNU versus methyl-CCNU plus 5-FU would demonstrate different responses in advanced colorectal carcinoma. Forty-nine patients have been evaluated, 14 receiving methyl-CCNU and 35 receiving 5-FU plus methyl-CCNU. One partial response has been seen with methyl-CCNU alone in a patient with liver metastasis. Thirteen partial responses have been noted in patients treated with the two-drug combination. There was a significant difference in the median survival of the responders versus the nonresponders for the two-drug group. Side effects were as expected: nausea and vomiting, leukopenia, and thrombocytopenia. Plasma carcinoembryonic antigen and urine arylsulfatase were measured in all patients and correlated well with response.

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The development of the antimetabolite 5-fluorouracil (5-FU) almost 20 years ago led to its successful clinical application in the treatment of large bowel cancer. A meager response rate of 15%–20% can be expected in patients with colorectal carcinoma when this drug is used as a single agent. These responses are usually only partial and transitory. Most alternative single agents have been ineffective in this frequently encountered malignant disease. The nitrosoureas and mitomycin C have approached the effectiveness of 5-FU, but no single drug has exceeded its effectiveness (1). It has been suggested that the most favorable nitrosourea for the treatment of colorectal cancer is methyl-CCNU (2).

Methyl-CCNU, BCNU (6), CCNU (7), streptozotocin (8), and chlorozotocin (9) are the nitrosoureas in clinical study in the US. Methyl-CCNU has been the most active compound tested in certain experimental solid tumors such as Lewis lung carcinoma, B16 melanoma, and C3H adenocarcinoma of the breast (3,4).

The active form and mechanism of action of methyl-CCNU are unknown. There is evidence that the nitrosoureas act through an alkylating mechanism (5). These compounds may also inhibit several key enzymatic processes leading to DNA synthesis (6). Other evidence indicates that the compounds may also have some mode of action by protein modification (7).

Initial clinical trials of methyl-CCNU began in 1971. Its efficacy in clinical cancer therapy has recently been reviewed (8). In 1973, Moertel (2) reported that methyl-CCNU appeared to be the best nitrosourea for treatment of large bowel carcinoma. This past year, Moertel et al reported a 44% response rate for methyl-CCNU in combination with 5-FU and vincristine versus a 20% response rate with 5-FU alone (1). Macdonald and his associates (9) recently reported a 43% response rate for this three-drug combination using weekly 5-FU without

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[4]Departments of Pathology (Dr. Posey) and Pharmacology and Medicine (Dr. Morgan), Louisiana State University Medical Center, New Orleans.

[5]The statistical evaluations by Dr. Gerald Metter, Group Statistician of the Central Oncology Group in Madison, Wis, are gratefully acknowledged.

*_Reprint requests to:_ Lynn E. Posey, PhD, Department of Pharmacology, Louisiana State University Medical Center, 1542 Tulane Ave, New Orleans, La 70112.

the loading dose. The Southwest Oncology Group has reported a 30% response rate for a combination of methyl-CCNU and 5-FU compared to a 14% rate for 5-FU alone in a mixed group of gastrointestinal cancers (10). A four-drug combination including BCNU, 5-FU, vincristine, and DTIC has elicited a 43% response rate in colorectal carcinoma according to Falkson et al (11). This paper reports our comparison of 5-FU plus methyl-CCNU versus methyl-CCNU alone in advanced colorectal carcinoma.

Arylsulfatase is a lysosomal enzyme often elevated in the tissues and urine of patients with a number of carcinomas: stomach, colorectal, breast, bladder, and melanoma (12). Our previous studies suggest that tissue and urine arylsulfatase plus plasma carcinoembryonic antigen (CEA) may be useful in evaluating prognosis in colorectal carcinoma (13).[6,7] In this study, we have followed response to therapy with plasma CEA and urine arylsulfatase.

MATERIALS AND METHODS

To date, we have studied 51 patients with advanced, unresectable colorectal cancer. Forty-nine of these patients are adequately evaluable. All patients studied had histologically confirmed, unresectable metastatic adenocarcinoma of the large bowel. Some patients had had prior therapy which will be discussed. Before a patient was entered in the study, there was a therapy-free observation period of 4 weeks following surgery, radiotherapy, or chemotherapy. Most of the patients were treated as outpatients. All were ambulatory, were in a reasonable nutritional state, and had an expected lifespan of > 2 months. Patients with one or more of the following contraindications were considered ineligible for entry into the study: leukopenia (< 3500 cells/mm^3), anemia (hemoglobin < 9 mg/dl or hematocrit $< 27\%$), thrombocytopenia ($< 100{,}000$ cells/mm^3), prior pelvic irradiation of > 5000 rads, or major surgery or chemotherapy within 4 weeks.

The first 28 patients were randomized to receive methyl-CCNU alone or a combination of methyl-CCNU plus 5-FU after stratification according to performance status, site of measurable malignant disease, and prior therapy with either antitumor drugs or radiation. However, after only one of 14 patients receiving methyl-CCNU alone had merely a stabilization of disease, all additional patients received the two drugs as initial therapy.

One group of patients received methyl-CCNU alone at a single oral dose of 175 mg/m^2 every 6 weeks. The other group received a loading dose of 5-FU at 12 mg/kg by iv push for 3 days and then once weekly with 175 mg/m^2 of methyl-CCNU given orally on Day 2; this course was repeated every 6 weeks. An attempt was made to titrate the leukocyte count in the absence of other toxic effects in the range of 2500–3500/mm^3. Patients who had an abnormal bilirubin (> 1.5 mg/dl) had a reduction in the methyl-CCNU dose to 125 mg/m^2, as well as a reduction of 5-FU to 8 mg/kg.

Pretreatment baseline evaluation consisted of a complete blood cell count, liver profile, urinalysis, plasma CEA, 24-hour urine for analysis of arylsulfatase activity, and appropriate radiologic workup including liver scans and, when indicated, bone scans and metastatic surveys. While receiving therapy, leukocyte and platelet counts were obtained weekly. Urine arylsulfatase and plasma CEA were determined monthly. Changes of a magnitude greater than three standard deviations of the assay were considered to be significant. Palpable, measurable lesions were evaluated weekly for the first 8 weeks, then every 2 weeks. Lesions evaluated radiologically were X-rayed every 2 months. Changes in liver scans were used as measurable disease. Abdominal tumor masses were measured by calipers.

The following criteria were used to judge response: (*a*) *complete response*—complete disappearance of all objective evidence of measurable disease with no new disease, (*b*) *partial response*—decrease of $\geq 50\%$ in the products of two diameters of measured lesions with no new lesions, (*c*) *no change*—a decrease of $< 50\%$ in the products of diameters of measured lesions with no new lesions, and (*d*) *progression*—an increase of $\geq 50\%$ in the products of measured lesions with or without new lesions. Patients were treated for at least 8 weeks, at which time they were evaluated for objective responses. All responses had to last at least 12 weeks.

If the measurable lesion was malignant hepatomegaly, a 30% reduction of the sum of the two measurements from the subcostal margin was considered to be a partial response.

Urine arylsulfatase was assayed according to the modified procedure[8] of Baum et al (14). Creati-

[6]Posey LE, Morgan LR, and Levin IA. Plasma CEA and urine enzyme activities in colorectal cancer. Presented at the Spring Meeting of the Southeast Section of the American Association of Clinical Chemists, Atlanta, Ga, Apr 1976.

[7]Posey LE, Morgan LR, Gillen LE, et al. A comparison of urine enzyme activities in colon-rectal cancer. Presented at the 3rd International Symposium on the Detection and Prevention of Cancer, NY, Apr 1976.

[8]Posey LE, and Morgan LR. Urine arylsulfatase activity in normal versus cancerous populations: manual method versus new automated method. Submitted to *Clinical Chemistry*, 1976.

nine assays were performed on all urine specimens to assure the completeness of the 24-hour urine collection. Plasma CEA was determined according to the method of LoGergo, Krupey, and Hansen (15).

RESULTS

The patient characteristics are described in table 1. Even though there were fewer patients receiving methyl-CCNU alone, randomization was still fairly effective. Although the sex ratios were slightly unbalanced, the two groups were quite similar with respect to age, grade of anaplasia, performance scale, status of the primary tumor, radiation therapy, and sites of malignant disease. The two groups were not balanced insofar as prior treatment is concerned. The combination group was overweighted with untreated patients. However, it is striking that the response rate was even higher in the treated versus the untreated patients with the two-drug combination. The numbers are small, however, and the differences are not statistically significant.

TABLE 1.—Patient characteristics and effectiveness of randomization

Characteristic	Patients receiving— Methyl-CCNU	5-FU + methyl-CCNU
Men/women	3/11	22/15
Age in yrs (mean)	64	63
Grade of anaplasia		
Low	1	3
Moderate	7	18
High	6	16
Performance scale (ECOG)*		
0	1	3
1	1	8
2	8	19
3	3	3
4	1	4
Status of primary tumor		
Resected	9	26
Unresected	5	11
Prior chemotherapy	8	14
Prior radiation therapy	3	2
Measurable malignant disease		
Liver	8	16
Abdomen	6	18
Lung	2	5
Other	1	1

*Performance: 0 = asymptomatic; 1 = symptomatic, but ambulatory; 2 = in bed < 50% of the time; 3 = in bed > 50% of the time; 4 = 100% bedridden.

TABLE 2.—Toxic reactions

Reaction	Methyl-CCNU (% of 14 patients)	Methyl-CCNU + 5-FU (% of 35 patients)
Nausea	66	65
Vomiting	45	57
Diarrhea	5	6
Stomatitis	15	20
Alopecia	5	30
Leukopenia nadir (cells/mm³)—		
< 3500 ≥ 2000	48	50
< 2000	30	25
Deaths from infection	1	0

Toxic reactions are listed in table 2. The predominant toxic reactions were nausea, vomiting, and myelosuppression. The vomiting is much more severe with the combination of methyl-CCNU and 5-FU than with methyl-CCNU alone. Vomiting was seen 6–8 hours after the administration of methyl-CCNU. Leukopenia was observed in two stages, with an early drop in the wbc count in the first 2 weeks followed by a more pronounced drop after 4–6 weeks (table 3). Thrombocytopenia (< 90,000 cells/mm³) occurred in 71% of the patients. Only one patient had a platelet count of 25,000/mm³. This patient had massive liver disease and developed pancytopenia while receiving therapy. She died from sepsis and hemorrhage and was considered the only drug-related death. 5-FU did not have any effect on the methyl-CCNU nadir.

When the two drug protocols are compared, there is an overall 37% response rate in our patients receiving two drugs as compared to only a 7% response rate in those receiving one drug. When the patients are evaluated with reference to responses and prior therapy (table 4), an interesting observation can be made. Of the 12 patients who had had prior treatment with 5-FU, six demonstrated a response to the two-drug combination. These 12 patients had received previous 5-FU therapy and either failed, responded then failed, or did not receive an adequate trial of therapy and could not be evaluated; however, when the combination of methyl-CCNU and 5-FU was initiated by our schedule, six of these patients exhibited dramatic responses. These six patients had had an adequate trial of 5-FU therapy and had responded and then failed. Only one patient responded to methyl-CCNU alone. This patient did have liver metastases. All other responders were receiving the combination.

TABLE 3.—Hematologic toxicity of 5-FU + methyl-CCNU

Toxic effect	Incidence (% of 35 patients)	Day of nadir	
		Mean	Range
Leukopenia (cells/mm³)			
Early			
< 3500 ≥ 2000	12	12	10–12
< 2000	1		
Middle			
< 3500 ≥ 2000	60	18	13–25
< 2000	25		
Late			
< 4000 ≥ 2000	45	39	28–54
< 2000	6		
Thrombocytopenia (cells/mm³)			
< 100,000 ≥ 50,000	55	22	15–32
< 50,000	18		

TABLE 4.—Summary of objective responses to methyl-CCNU + 5-FU in colorectal cancer*

Mode of treatment	No. of patients	Objective response (%)
No prior treatment		
Methyl-CCNU	6	17
5-FU + methyl-CCNU	21	33
Prior treatment with methyl-CCNU		
5-FU + methyl-CCNU	2	0
Prior treatment with 5-FU		
Methyl-CCNU	8	0
5-FU + methyl-CCNU	12	50

*All patients at 8 wks were judged to have either partial responses or progression.

TABLE 5.—Changes in urine arylsulfatase and plasma CEA in patients with colorectal cancer who responded to 5-FU + methyl-CCNU

Patient No.	Response	Disease stage	Urine arylsulfatase (normal, 2.6–7.3 units/day)		Plasma CEA (normal, < 2.5 ng/ml)	
			Before	After	Before	After
1	Complete	D	8.5	7.2	15.1	3.2
2	Partial	D	21.0	11.0*	>50	13.0*
3	Partial	D	23.0	8.1*	>50	20.0*
4	Partial	D	13.0	6.1*	42.0	20.0*
5	Partial	D	26.3	8.9*	>50	12.7*
6	Partial	D	6.7	7.1	10.0	4.7*
7	Partial	D	8.7	7.2	3.5	2.3
8	Partial	D	10.9	6.5*	>50	12.3*
9	No change	D	15.1	13.2	35	40

*Significantly different from the pretreatment level.

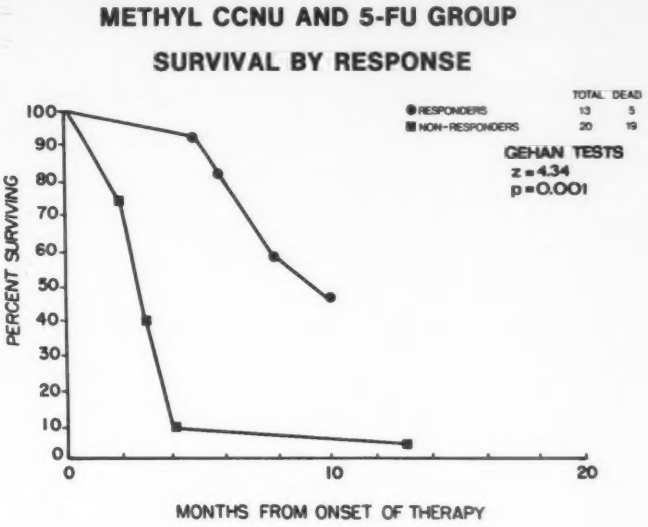

FIGURE 1.—Comparison of survival of responders and nonresponders for patients treated with both 5-FU and methyl-CCNU.

Although the patients adequately evaluated are few, a distinct difference in the survival between responders and nonresponders can be seen for the two-drug combination. In figure 1, responders are compared to nonresponders with significantly longer survival for the responders (Gehan test: Z = 4.34, P = 0.001). The median survival times were 9.3 versus 3 months, respectively. No significant difference was seen in survival by sex.

Table 5 shows changes in urine arylsulfatase activity and plasma CEA in nine patients who responded to 5-FU and methyl-CCNU. Complete data were not available for the remaining four responders. In all patients who responded, the urine arylsulfatase activity decreased or remained about the same after therapy, as did the plasma CEA. Similar results have been reported previously by Morgan et al (13) for patients who responded to 5-FU alone. In patients who did not respond, both the urine arylsulfatase activity and the plasma CEA increased.

DISCUSSION

We have noted an increased response in large bowel cancer to the combination of 5-FU and methyl-CCNU as compared to either drug alone. Moreover, there is a significant increase in survival time for the responders compared to the nonresponders for the two-drug combination. There is progressive impairment of the bone marrow with methyl-CCNU therapy, demonstrated by a delay in bone marrow regeneration. The double nadir of leukopenia observed has also been noted previously by Moertel (2) not only for methyl-CCNU but also for CCNU and BCNU in therapy for large bowel cancer. We observed a progressive myelosuppression due to methyl-CCNU such that after three or four courses of therapy, the drug can only be given every 8–10 weeks instead of every 6 weeks. Those patients with liver disease demonstrated lower leukocyte and platelet nadirs when given the full dose of the drug; therefore, all patients with abnormal bilirubin should have a reduction in both methyl-CCNU and 5-FU doses. Though not indicated above, the patients who responded the best were patients with liver disease.

We agree with others (1,9,10) that methyl-CCNU, when added to 5-FU, is an effective drug combination. We have noted that responses could be elicited by adding methyl-CCNU to 5-FU in patients who had previously responded and then failed with 5-FU alone. Similarly, Cedermark et al (16) found that response to methyl-CCNU was not related to previous response to 5-FU treatment. Moertel (17), however, has previously reported that the effectiveness of methyl-CCNU is substantially reduced when the drug is administered to patients who have previously failed while receiving 5-FU.

It is interesting and important to compare the responders and the nonresponders with untreated patients. Moertel (17) has reported that patients with untreated metastatic colon cancer have a median survival of approximately 6 months. This is not too dissimilar from the responding group and is longer than the nonresponding group. This may indicate that the nonresponding group is adversely affected by methyl-CCNU and 5-FU therapy.

We continue to be impressed with the use of urine arylsulfatase activity and plasma CEA as biochemical indices for monitoring response in the treatment of cancer of the large bowel. As we have noted previously (13), urine arylsulfatase activity decreases with regression of disease. As the extent of disease increases, so does the urine arylsulfatase activity. These changes were found to correlate well with changes seen in plasma CEA and are of value in following response to therapy.

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Characterization and Responsiveness of the Madison 109 Lung Carcinoma to Various Antitumor Agents [1,2]

Thomas A. Marks,[3,4,*] Richard J. Woodman,[3] Ruth I. Geran,[5] Leonard H. Billups,[3] and Russel M. Madison [3,6]

SUMMARY

The Madison 109 (M109) tumor was discovered in 1964 in the lung of a BALB/c mouse. This experimental carcinoma is maintained in vivo by sc passage in the right axillary region. When implanted im (5×10^5 cells) into the right hind leg of BALB/c mice for testing, the primary tumor progresses with metastases to the lung, spleen, and liver. The metastases to the lung are visible within 3 weeks and result in the death of the host in about 35 days after tumor implant. Implantation of a lung nodule is tumorigenic and lethal. Pyran polymer therapy delayed the appearance of lung metastases, inhibited the growth of the primary tumor, and significantly increased the lifespan of BALB/c mice inoculated with the M109 tumor. No spontaneous regression has been observed and very few "no takes" have occurred in untreated BALB/c mice inoculated with at least 500 M109 cells. Of the 82 agents tested so far, the M109 model has selected active agents such as actinomycin D, adriamycin, daunorubicin, DNA, procarbazine, and pyran polymer. It has not shown sensitivity as tested to several standard therapeutic agents including cytosine arabinoside, BCNU, hydroxyurea, mechlorethamine, melphalan, triethylenemelamine, and vincristine.

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Solid tumors represent the chief cause of human cancer mortality (1). Such tumors have been highly resistant to standard chemotherapy and thus there is a need for the development of a slow-growing experimental solid tumor which is not overly sensitive to those agents that have shown some success against human malignancies (2).

In the hope of providing an additional tool capable of selecting new agents for the treatment of human solid malignancies, the staff of Microbiological Associates studied the drug sensitivity of various slow-growing solid murine tumors. During this study, the Madison 109 (M109) lung carcinoma became of interest when early tests indicated that this tumor was sensitive to pyran polymer, an agent which was not active in murine leukemia models such as L1210 and P388. Interest was heightened when it was found that the tumor appeared to be relatively resistant to such standard chemotherapeutic agents as cytosine arabinoside (ara-C), BCNU, melphalan, etc.

Preliminary communications (3,4) have described the potential benefit of the different pattern of sensitivity observed in the M109 model and other investigators (5) have recently reported on their in vitro as well as in vivo investigations with this tumor system. The effect of actinomycin D, adria-

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[3]Microbiological Associates, Bethesda, Md.

[4]*Present address:* Chemistry and Life Sciences Division, Research Triangle Institute, Research Triangle Park, NC.

[5]DCT, NCI, Bethesda, Md.

[6]We wish to thank Dr. James E. Ollman for preparing the oxidized polysaccharides and the hydrolyzed pyran polymer. We are grateful to Dr. D. S. Breslow, Hercules, Inc, for information concerning the chemistry of pyran polymer. The helpful suggestions concerning the statistical evaluation of our data provided by C. Andrew Clayton, Statistics Research Division, Research Triangle Institute, are most appreciated.

[*]*Reprint requests to:* Thomas A. Marks, PhD, Chemistry and Life Sciences Division, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709.

mycin, daunorubicin, and DNA on the M109 tumor has been covered in more detail in another publication (6). This report describes some of the basic biologic characteristics of the M109 tumor as well as the responsiveness of this model to a variety of therapeutic agents.

MATERIALS AND METHODS

The M109 lung carcinoma has been maintained in vivo by sc transplantations in BALB/c mice every 14–21 days. For transplantation, the excised sc tumor was dissected aseptically, denuded of necrotic tissue, and reduced to a suspension, consisting of predominantly single cells, by employing a tissue press or by forcing the tumor fragments through a screen (Tyler standard screen, 0.0116-inch opening, 48 meshes/inch). Hanks' balanced salt solution was utilized as the suspending vehicle and counts of intact nucleated cells were determined with a hemocytometer employing the trypan blue dye-exclusion technique. M109 cells were grown in vitro by utilizing the techniques outlined by Madden and Burk (7). For histologic examination, tumor tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, cut at 4–6 μm, and stained with hematoxylin and eosin. The mitotic index was determined by counting ten fields in histologic sections at 400× with the aid of an ocular grid and expressed as the average number of mitoses per hundred carcinoma cells.

For in vivo testing, the M109 tumor brei was inoculated im (Day 0) into the right hind leg of male BALB/c mice, employing a concentration of 5×10^5 tumor cells (except where indicated) in 0.1 ml of Hanks' balanced salt solution. Each test group contained eight mice with 16 untreated control mice (except where indicated). Treatment began 24 hours after tumor inoculation utilizing the schedules specified. Each drug was tested at four or more dose levels. Pyran polymer was the positive control compound. All agents were injected ip in a volume of 0.01 ml/g of body weight. Only male BALB/c mice (provided by the Mammalian Genetics and Animal Production Section, Developmental Therapeutics Program [DTP], DCT, NCI), ranging between 8 and 12 weeks old and weighing between 19 and 28 g, were used. Tumor dimensions were determined by measuring, with a vernier caliper, the mediolateral and anterioposterior axes. Tumor weight was then estimated from these dimensions (8). The India ink method of Wexler (9) was utilized to facilitate the identification of lung metastases. The median survival time (MST) was calculated as previously described (6). The increase in lifespan (ILS) was calculated by dividing the MST of the treated mice by the MST of the untreated control mice, subtracting 1, and multiplying by 100. The Wilcoxon (Mann-Whitney) rank sum test was utilized to determine at which level of significance the null hypothesis of equal MSTs for untreated control and treated groups could be rejected.

The oxidized polysaccharides employed in this study were prepared by Dr. J. E. Ollmann (Organic Chemistry Division, Microbiological Associates) from polysaccharides purchased from the Sigma Chemical Co (alginic acid, amylose, cellulose, dextran, and polygalacturonic acid), essentially as outlined by Claes et al (10). Dr. Ollmann also prepared hydrolyzed pyran polymer by stirring pyran polymer for 70 hours in 0.2 N NaOH (at a 100:1 ratio [vol/wt]). After adjusting to pH 4 with 1 N HCl, the solution was dialyzed, lyophilized, redissolved in H_2O, and passed through a Dowex 50W-X8 column. The fractions containing the hydrolyzed pyran polymer were then lyophilized.

Calf thymus DNA and herring sperm DNA were also purchased from Sigma Chemical Co. Mithramycin was a generous gift from Dr. Keith Jenson, Pfizer, Inc, and the pyran polymers were graciously provided by Dr. D. S. Breslow of Hercules, Inc. All other agents used in this study were obtained from DTP, DCT, NCI. The names of the agents with confirmed, unconfirmed, or borderline activity are given in List A. The names of the agents that were negative as tested are given in List B.

RESULTS

The sc inoculation of M109 tumor cells (brei) into BALB/c mice resulted in the growth of a nodular unencapsulated mass. In some areas the tumor grew in cords of proliferating cells separated by a delicate capillary stroma (figs 1–4). In other areas, the cells grew in nests compressing but seldom infiltrating the surrounding sc connective tissue. Focal areas of necrosis and inflammatory cell infiltrates were frequently observed in most of the tumors examined. The proliferating cells contained abundant eosinophilic, finely vacuolated cytoplasm with indistinct cytoplasmic borders. The nuclei varied from round to oval and contained two to three nucleoli. The chromatin was granular and fairly evenly distributed. Many cells contained nuclei which were vesiculated with prominent nucleoli. Occasionally the cells contained nuclear cytoplasmic invaginations. The mitotic index was 2.4 mitoses per hundred carcinoma cells.

Metastatic lesions in the lungs were often located just beneath the pleura. Several small blood vessels

associated with bronchioles were plugged with tumor emboli which often extended into surrounding lung parenchyma forming circumscribed nodules. The cellular morphology of the metastatic lesions was identical to the primary sc tumor which was morphologically a poorly differentiated carcinoma.

The data in table 1 indicate that the survival time of the BALB/c mouse inoculated with the M109 tumor decreased as the number of cells injected was increased. However, there was a relatively small decrease in host survival when the number of intact tumor cells in the inoculum was increased from 5×10^3 to 5×10^4 cells. When only 50 cells were inoculated, more than half of the mice did not develop palpable tumor by Day 85 and thus can be considered as "no takes." This experiment was originally set up to measure the effect of standing on tumorigenicity. Thus, the tumor was prepared and, based on the number of carcinoma cells which excluded trypan blue, adjusted to the concentrations indicated, which were then each divided into two parts. One part of each dilution was left on ice (4°C) while the other part was allowed to come to room temperature (22°C). The tumor on ice was immediately injected into ten mice, with additional tumor inoculated at 1-hour intervals, up to 4 hours, into groups of ten mice. The tumor left at room temperature was inoculated into ten mice, 1 hour after removal from the ice bucket. Although the percentage of intact tumor cells decreased from 68.5% to 40.6% (4°C) over the 4 hours and to 56.0% after 1 hour at 22°C, there appeared to be no appreciable differences in the survival time with the various inoculum levels. Therefore, the six groups at each inoculum level were combined giving the results shown in table 1. The results obtained in this experiment for 5×10^5 M109 cells are very close to the "average" (35.9 days) and "median" (36.8 days) MST (32–39.8-day range) obtained for the control mice in the nine chemotherapy tests in which this inoculum level was employed.

Eighty-two compounds were tested for survival time increases in the M109 carcinoma model including many of the antitumor agents which are considered to be clinically active (1). Testing of each compound was similar to that carried out for actinomycin D, etc, described in detail in a previous publication (6). When sufficient compound was available, the active agents were tested at least a second time. Since the primary aim of the chemotherapy experiments was to test as many known antitumor agents as possible in order to get an idea as to the potential usefulness of the M109 tumor model, an extensive investigation of most of the drugs was not carried out.

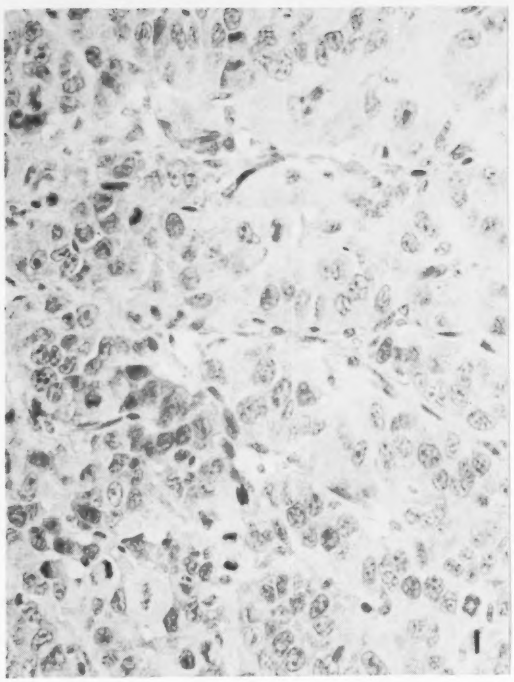
Nine percent (seven agents) of the compounds tested against M109 produced confirmed activity (table 2); 28% (23 agents) had unconfirmed activity (table 3); 12% (ten agents) showed borderline activity (table 4); and 51% (42 agents) were inactive as tested (*see* List B). Actinomycin D was active on several schedules, including treatment every other day (Days 1–17), every third day (Days 1–25), and daily (Days 1–9). On the other hand, tilorone, an agent which was not active on a daily, Days 1–9 schedule, was active (table 3) when given every fourth day (Days 1–33). Daunorubicin did not produce activity in this study (table 2) until the dose was reduced significantly below that which has produced optimal activity (1.0–2.5 mg/kg; daily, Days 1–9) against the experimental leukemias employed in this laboratory (6). Thus, it is possible that some of the compounds which have not shown significant activity may give better results if various schedules or lower doses are tested.

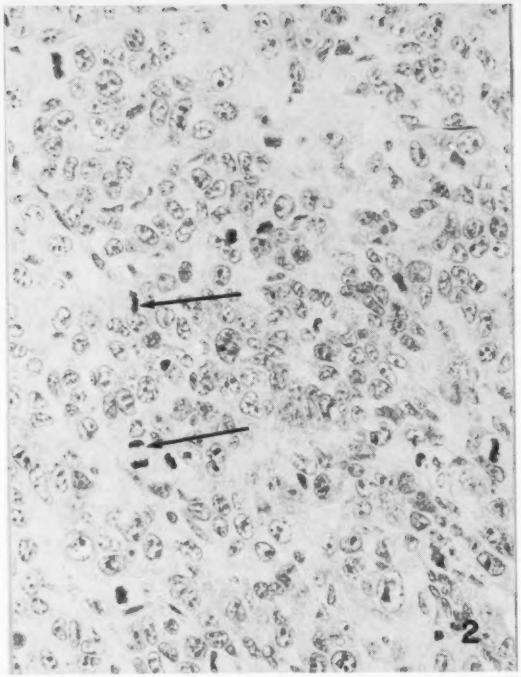
Adriamycin and procarbazine are two other agents which have been successfully employed in the clinics and have had confirmed activity against M109. However, other clinically active compounds such as ara-C, BCNU, hydroxyurea, mechlorethamine, melphalan, triethylenemelamine, vincristine, etc, did not show significant activity against M109 at concentrations and schedules which have shown activity against other experimental tumor systems.

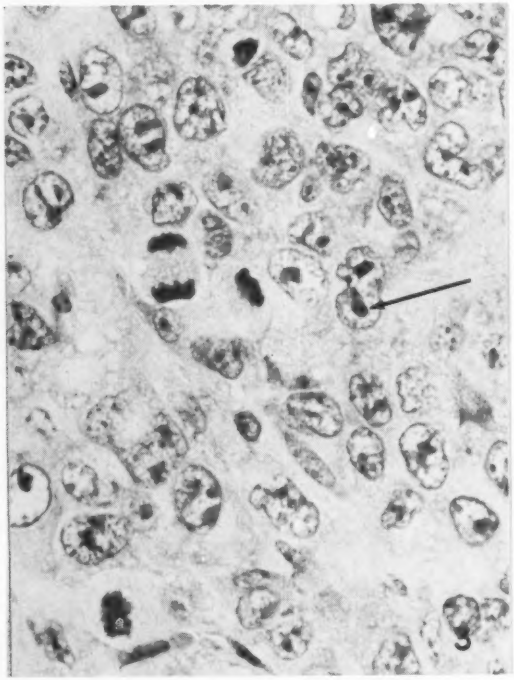
Pyran polymer (a polymer of divinyl ether and maleic anhydride) was used as the positive control compound for the model and it was tested more thoroughly than any other agent. This compound was active on every schedule investigated including Day 1 only. Its activity in this model was reproducible. In 12 of the 13 experiments in which pyran polymer was tested on a daily, Days 1–9 schedule (ip), the optimum dose (40–50 mg/kg/injection) produced an ILS of at least 33% (range, 33%–72%; average, 53%).

Pyran polymer treatment delayed the onset of detectable M109 tumor and also slowed the early rapid tumor growth (fig 5). Palpable tumor was detected in the treated animals during the period chemotherapy was being administered, but about 2 days after tumor was measurable in the untreated controls. In another experiment, pyran polymer treatment (50 mg/kg, Days 1–9) was able to produce a significant delay (2–3 weeks) in the appearance of lung metastases as compared to untreated BALB/c mice inoculated with the M109 tumor.

Pyran polymer is solubilized by adding H_2O and adjusting the pH to 7 with dilute NaOH, a process which most likely hydrolyzes the maleic anhydride residues to carboxy groups. In order to determine if the degree of hydrolysis could affect the results,







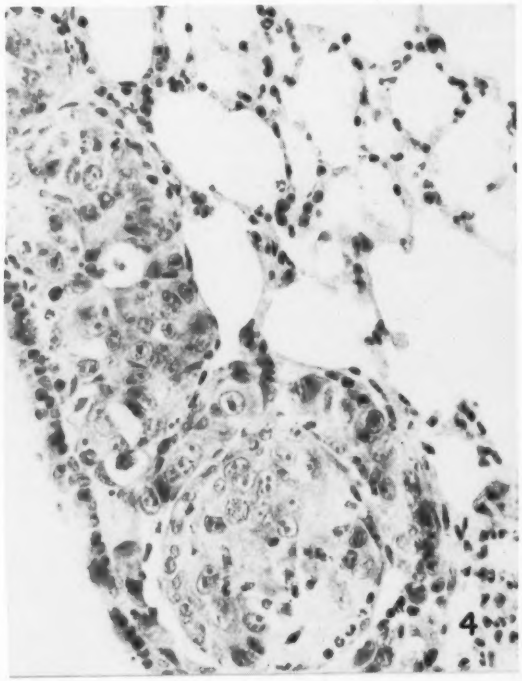


FIGURE 1.—Sc transplant of the M109 mouse lung carcinoma (× 480). (Note arrangement of cells in nests or cords separated by fine fibrovascular stroma).

FIGURE 2.—Note numerous mitotic figures (arrows) and nuclear pleomorphism (× 480).

FIGURE 3.—A higher magnification showing cells with indistinct cytoplasmic borders, large vesiculated nuclei with nucleoli which are often multiple (arrow) (× 800).

FIGURE 4.—Metastatic foci in lung extending from a tumor emboli in a peribronchiolar blood vessel (× 128).

TABLE 1.—Effect of inoculum size on survival time of mice inoculated with the M109 lung carcinoma (male BALB/c mice [60 mice/test group] were inoculated im with the No. of M109 tumor cells indicated)

Inoculum size (cells)	Mean survival time* (days)	MST† (days)
5×10^1	"53.4 ± 8.6"‡	—
5×10^2	51.2 ± 7.2	51.3
5×10^3	42.4 ± 6.1	42.3
5×10^4	41.8 ± 4.2	42.2
5×10^5	35.7 ± 5.0	36.3

*± 1 SD of the mean.

†MST calculated according to a formula referenced in Methods section.

‡Since only 27 of 60 mice died by Day 85 at this inoculum level, this result is the mean of those mice which did die by Day 85.

TABLE 2.—Confirmed active agents against the M109 carcinoma*

Agent	Schedule	Apparent optimal dose (mg/kg)	MST of controls (days)	MST of treated mice (days)	ILS (%)	Level of significance
Actinomycin D	Every 2 days, Days 1–17	0.1	38.0	52.0	37	0.05
	Every 3 days, Days 1–25	0.08	38.0	52.0	37	0.05
Adriamycin	Daily, Days 1–5	1.5	31.0†	42.3	36	0.01
Calf thymus DNA‡	Daily, Days 1–9	0.6	32.3†	41.3	27	0.001
Daunorubicin	Daily, Days 1–5	0.5	31.0†	43.0	38	0.025
Herring sperm DNA	Daily, Days 1–5	20	31.0†	46.0	48	0.01
Procarbazine	Daily, Days 1–9	200	36.8	49.0		0.05
	Daily, Days 1–9	25	34.0	45.3	33	0.01
Pyran polymer§	Daily, Days 1–9	50	36.0	62.0	72	0.001

*Agents which produced an ILS ≥ 25% in ≥ 2 experiments.

†Mice in this experiment were inoculated with 10^5 tumor cells.

‡In this experiment each test group contained 10 mice with 20 untreated control mice.

§In this experiment each test group contained 6 mice with 12 untreated control mice.

TABLE 3.—Agents with unconfirmed activity against the M109 carcinoma*

Agent	Schedule	Apparent optimal dose (mg/kg)	MST of controls (days)	MST of treated mice (days)	ILS (%)
Alginic acid	Daily, Days 1–9	50	32.0	40.3	25
BCG	Day 1 only	250	35.0	45.0	29
Bleomycin	Every 4 days, Days 1–9	10	34.0	48.0	41
Chlorambucil	Daily, Days 1–9	6	34.0	44.8	31
1-Chloro-2,4-dinitrobenzene	Daily, Days 1–9	20	31.0†	43.0	38
2-Chloro-1,3-dinitrobenzene	Every 2 days, Days 1–17	80	31.0†	41.0	32
2-Chloro-1,4-dinitrobenzene	Every 2 days, Days 1–17	160	31.0†	40.3	30
4-Chloro-1,2-dinitrobenzene	Every 2 days, Days 1–17	40	31.0†	41.3	33
Chromomycin A_3	Daily, Days 1–9	0.064	33.0	43.0	30
Colchicine	Daily, Days 1–9	0.48	33.0	42.0	27
DTIC	Daily, Days 1–9	100	34.8	49.0	40
Glucosamine	Daily, Days 1–9	1600	30.3‡	38.0	25
Guanazole	Daily, Days 1–9	400	32.0	42.0	31
Oxidized amylose polyacid	Daily, Days 1–9	50	32.0	42.3	32
Oxidized cellulose polyacid	Daily, Days 1–9	200	32.0	43.0	34
Oxidized dextran polyacid	Daily, Days 1–9	200	32.0	44.0	37
Oxidized polygalacturonic acid	Daily, Days 1–9	50	32.0	41.8	30
Pactamycin	Daily, Days 1–9	1.2	33.0	45.7	38
Prednisone	Daily, Days 1–9	20	34.0	43.0	26
Pyran polymer (hydrolyzed)	Daily, Days 1–9	40	34.8	47.3	35
Streptonigrin	Daily, Days 1–9	0.04	33.0	44.0	33
ThioTEPA	Daily, Days 1–9	20	34.8	46.8	34
Tilorone	Every 4 days, Days 1–33	100	34.0	43.0	26

*Agents which produced an ILS ≥ 25% at ≥ 1 dose on 1 test.
†Mice in this test were inoculated with 10^5 cells.
‡Mice in this test were inoculated with 10^6 cells.

TABLE 4.—Agents with borderline activity against the M109 carcinoma*

Agent	Schedule	Apparent optimal dose (mg/kg)	MST of controls (days)	MST of treated mice (days)	ILS (%)
BCNU glycoside	Day 1 only	14	36.0†	44.8	24
Dibromodulcitol	Daily, Days 1–9	50	34.8‡	43.0	24
Ellipticine	Daily, Days 1–9	4	33.0	40.0	21
5-Fluorouracil	Daily, Days 1–9	15	34.0	42.0	23
Levamisole	Daily, Days 1–9	1.5	34.0	41.8	22
Methotrexate	Daily, Days 1–9	0.15	34.0	42.0	23
Mithramycin	Daily, Days 1–9	0.2	36.8	44.0	20
Pectin	Daily, Days 1–9	100	32.0	39.3	22
Poly I:Poly C	Daily, Days 1–9	10	35.0‡	42.5	23
		2.5			
Vinblastine	Daily, Days 1–9	1.0	36.8	44.8	21
		0.1	32.0	39.0	

*Agents which produced an ILS ≥ 20% but < 25%.
†Mice in this experiment were inoculated with 10^6 cells.
‡Mice in this experiment were inoculated with 10^5 cells.

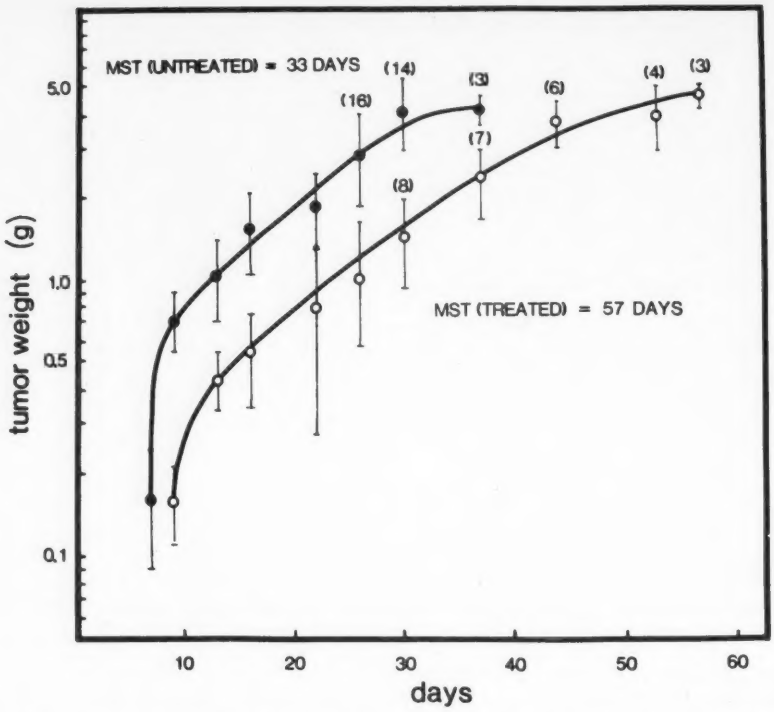


FIGURE 5.—Effect of pyran polymer (50 mg/kg, ip, Days 1–9) on M109 lung tumor weight (inoculated im into the right hind leg of male BALB/c mice). Each point represents an average of the tumor weights of 8 (treated) or 16 (untreated) mice, calculated from measurement of tumor diameters with vernier calipers (ref 8). Vertical lines give the SD of the mean. The No. of mice alive is given in parentheses. MST = calculated median survival time.

TABLE 5.—Effect of molecular size on the activity of pyran polymer against the M109 carcinoma*

Reduced specific viscosity value for pyran polymer	Dose					
	50 mg/kg			25 mg/kg		
	MST† (days)	ILS (%)	Level of significance	MST† (days)	ILS (%)	Level of significance
0.044‡	43.0	19	0.05	33.0	0	—
0.08‡	47.0	31	—	47.0	31	0.025
0.014‡	40.0	11	—	53.0	47	0.01
0.21‡	49.0	36	0.001	52.0	44	0.001
0.37‡	56.0	56	0.05	50.0	39	0.01
0.55‡	50.0	39	0.001	47.3	31	0.01
0.38§	62.0	72	0.001	57.0	58	0.001

*Polymers injected daily, Days 1–9; each test group contained 6 mice with 12 untreated control mice.

†Calculated MST of controls was 36.0 days.

‡Narrow molecular weight range pyran polymer with a mean reduced specific viscosity as indicated.

§Broad molecular weight range pyran polymer with a mean reduced specific viscosity of 0.38.

the effect of extensive hydrolysis (*see* Methods) on pyran polymer activity against M109 was tested. The results (table 3) obtained with "hydrolyzed pyran polymer" indicate that extensive hydrolysis does not significantly reduce the activity of this macromolecule.

Table 5 shows the results of an experiment designed to determine the effect of the molecular size of pryan polymer on the therapeutic response. Although significant activity was obtained in the 0.08–0.55 reduced specific viscosity (1% solution in 1 N NaOH) range, the standard pyran polymer, which has a wide range of molecular sizes with a mean of 0.38, produced the greatest ILS in the experiment.

DISCUSSION

The progress against hematologic malignancies which has been achieved in the last several years with chemotherapy has as yet not been translated sufficiently to the common solid tumors that are the chief cause of human cancer deaths (11). In order to attempt to improve the drug treatment of human solid tumors, it seems advisable to have available additional experimental murine models that will select drugs other than the "standard" chemotherapeutic agents. Preliminary studies indicate that the M109 lung carcinoma may be such an experimental tumor model, and therefore its biologic characteristics are described here.

In November 1964, Dr. Russel M. Madison discovered the M109 tumor in the lung of an 18-month-old BALB/c mouse. This mouse was one of a test group at Microbiological Associates which had been injected with a cell-free extract of a human testicular tumor. However, there was no additional evidence to identify this cell-free extract as a causative factor of M109. Thus, the tumor was considered to have developed "spontaneously." The M109 tumor is a highly stable transplantable tumor which kills all recipient untreated mice within a fairly narrow range of time after inoculation, with no evidence of spontaneous regression. When inoculated (im) as a brei (5×10^5 cells) into the hind leg of a BALB/c mouse, the tumor continues to grow, producing lung metastases visible to the naked eye in about 3 weeks and the death of the host in about 5 weeks. Metastases appear initially as single nodules which then increase in number. At death the lungs are so infested with tumor that India ink treatment shows less black (normal tissue) than white (metastases) on the lung surface. The tumorigenicity of the metastatic foci in the lungs was confirmed by killing mice which had been inoculated with the M109 tumor 3 weeks previously, removing their lungs, and inoculating the same (sc) into normal BALB/c mice (12). The resultant tumor growth led to the death of the inoculated mice. Removal of even a single tumor nodule from the surface of the lung, upon reinoculation, caused tumor growth followed by death of the host.

The M109 primary tumor often becomes highly necrotic after the third week of growth. Also, the tumor frequently becomes ulcerated about 4–5 weeks after tumor inoculation. Therefore, the tumor line is best maintained by transplanting biweekly. The M109 has been successfully stored under liquid nitrogen for several years and on various occasions a sample of the tumor has been thawed and successfully reinoculated into BALB/c mice. The tumor has also been maintained in vivo by transplanting into BALB/c × DBA/2 F_1 ($CD2F_1$) hybrids. Attempts to transplant the tumor into C57BL/6 or C3H mice were not successful. M109 tumor cells were maintained in vitro and, upon inoculation of these cells into BALB/c or $CD2F_1$ mice, tumors developed which subsequently caused the death of the host.

The histology and growth characteristics of the M109 have been consistent for > 10 years. The fact that the tumorigenicity of M109 cells removed from a BALB/c mouse was not significantly changed after 4 hours at 4°C or 1 hour at room temperature provides support for the premise that the tumor is stable under the usual tumor-transplant conditions. M109 is easily maintained both in vivo and in vitro. As few as 50 viable cells have been shown to produce visible tumor growth with the death of the BALB/c host. No spontaneous regression has been observed and very few, if any, "no takes" have occurred in untreated BALB/c mice inoculated with ≥ 500 M109 cells. Although extensive in vitro testing was not carried out in this investigation, Schultz et al (5) have performed such studies with M109 tumor cells.

The M109 tumor is not overly sensitive to single-agent therapy. This is borne out by the fact that, of the seven agents which have produced confirmed activity, so far only pyran polymer has produced an ILS of > 50% when given alone. Freedom from over-sensitivity could permit such a model to differentiate between structurally related agents where an overly sensitive model is not capable of ranking a curative class of compounds.

Although the tumor responds to many of the agents which have shown activity in other experimental models, M109 has several characteristics and capabilities which indicate that it is not simply duplicating the effects seen with other tumor systems. This becomes evident when one compares the M109 tumor with a similar tumor model—the Lewis

lung carcinoma. The latter tumor has been extensively utilized for primary and secondary testing of chemotherapeutic agents (13,14). These two lung carcinomas do not always respond similarly to the same compounds. For example, actinomycin D, adriamycin, daunorubicin, and procarbazine were confirmed active against M109 but have not shown activity against the Lewis lung tumor.[7] Similarly, ara-C, BCNU, melphalan, and methyl-CCNU are active against the Lewis lung carcinoma[7] but did not show activity in this study against the M109 lung carcinoma. However, pyran polymer is active against both of these experimental lung carcinomas (5,15).

In addition to DNA and pyran polymer, other high molecular weight agents were able to significantly increase the lifespan of BALB/c mice inoculated with the M109 tumor. Some of these compounds (eg, bacillus Calmette-Guérin [BCG]) have been shown to affect the immune response in various ways (16), as have relatively smaller molecular weight M109-active agents such as tilorone (17) and dinitrochlorobenzene (16). The involvement of the macrophage in pyran polymer therapy of the M109 tumor was the subject of a recent publication (5). The results indicate that the M109 model is useful in the study of nonspecific activation of macrophages as well as transplantation or tumor immunity.

The M109 tumor meets many of the characteristics desirable for a solid tumor model, as outlined by Skipper and Schabel (18). The tumor is transplantable, manipulatable, and reproducible with respect to growth rate of the primary. The tumor has a growth rate of from 1 day to several days when small as compared to longer times when advanced. The tumor can be employed in both early and advanced stages and chemotherapy can be used as an adjuvant to other therapeutic modalities, in addition to using chemotherapy alone. The tumor metastasizes widely with uniform lethality to the host at a reproducible period after a standard inoculum. The effect of therapy on lung metastases can be measured at least semiquantitatively giving one a third measurable treatment parameter for the M109 tumor model, in addition to ILS and primary tumor size. Thus the M109 lung carcinoma has biologic characteristics which indicate that it warrants trial as an experimental tumor model to aid in the selection of agents for human solid-tumor therapy.

[7]Wood HB, Jr. Chemical structures of current interest to the Division of Cancer Treatment: drugs with clinical activity. Bethesda, NCI, 1977, pp 10–13.

LIST A: "active" (confirmed, unconfirmed, and borderline) compounds

Actinomycin D: NSC-3053; dactinomycin
Adriamycin: NSC-123127; *L-lyxo*-hexopyranoside, 3β-glycolyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1α-naphthacenyl-3-amino-2,3,6-trideoxy-, α-, hydrochloride
Alginic acid: NSC-50801; polymannuronic acid, sodium salt
BCG: NSC-B99832; bacillus Calmette-Guérin
BCNU glycoside: NSC-114460; glucopyranose, 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-, tetraacetate (ester), D-
Bleomycin: NSC-125066
Calf thymus DNA: NSC-196526
Chlorambucil: NSC-3088; butyric acid, 4-[*p*-[bis(2-chloroethyl)-amino]phenyl]-
1-Chloro-2,4-dinitrobenzene: NSC-6292
2-Chloro-1,3-dinitrobenzene: NSC-21276
2-Chloro-1,4-dinitrobenzene: NSC-154554
4-Chloro-1,2-dinitrobenzene: NSC-119343
Chromomycin A_3: NSC-58514
Colchicine: NSC-757; benzo[α]heptalen-9(5*H*)-one, 7-acetamido-6,7-dihydro-1,2,3,10-tetramethoxy-
Daunorubicin: NSC-82151; L-*lyxo*-hexopyranoside, 3β-acetyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxo-1α-naphthacenyl-3-amino-2,3,6-trideoxy-, α-, hydrochloride
Dibromodulcitol: NSC-104800; galactitol, 1,6-dibromo-1,6-dideoxy-
DTIC: NSC-45388; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-
Ellipticine: NSC-71795; 6*H*-pyrido[4,3-*b*]carbazole, 5,11-dimethyl-
5-Fluorouracil: NSC-19893
D-Glucosamine: NSC-758; D-glucose, 2-amino-2-deoxy-, hydrochloride
Guanazole: NSC-1895; 1,2,4-triazolidine, 3,5-diimino-
Herring sperm DNA: NSC-184725
Levamisole: NSC-177023; imidazo[2,1-*b*]thiazole, 2,3,5,6-tetrahydro-6-phenyl-, hydrochloride
Methotrexate: NSC-740; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-
Mithramycin: NSC-24559
Oxidized amylose polyacid: NSC-212573; poly[oxy(carboxymethylene)oxy[1-carboxy-2-(hydroxymethyl)-1,2-ethanediyl]]; molecular weight about 1×10^5
Oxidized cellulose polyacid: NSC-212574; poly[oxy(carboxymethylene)oxy[1-carboxy-2-(hydroxymethyl)-1,2-ethanediyl]]; molecular weight about 1×10^6
Oxidized dextran polyacid: NSC-212575; poly[oxy(carboxymethylene)oxy(1-carboxy-1,2-ethanediyl)]; molecular weight about 1×10^5
Oxidized polygalacturonic acid: NSC-212576; poly[oxy(carboxymethylene)oxy(1,2-dicarboxy-1,2-ethanediyl)]; molecular weight about 1×10^4
Pactamycin: NSC-52947
Pectin: NSC-4063; galacturonic acid, polymer; polygalacturonic acid
Poly I:Poly C: NSC-120949; 5′-inosinic acid, polymers, complex with 5′-cytidylic acid polymers (1:1)
Prednisone: NSC-10023; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-
Procarbazine: NSC-77213; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, hydrochloride
Pyran polymer: NSC-46015; pyran-2-succinic anhydride, 4,5-dicarboxytetrahydro-6-methyl-, anhydride, polymers
Streptonigrin: NSC-45383; picolinic acid, 5-amino-6-(7-amino-5,8-dihydro-6-methoxy-5,8-dioxo-2-quinolyl)-4-(2-hydroxy-3,4-dimethoxyphenyl)-3-methyl-
ThioTEPA: NSC-6396; phosphine sulfide, tris(1-aziridinyl)-

Tilorone: NSC-143969; fluoren-9-one, 2,7-bis[2-(diethylamino)ethoxy]-, dihydrochloride
Vinblastine: NSC-49842; vincaleukoblastine, sulfate (1:1), monohydrate

LIST B: inactive (as tested) compounds

Amphotericin B: NSC-527017
Amylose: NSC-225104
Ara-C: NSC-63878; cytosine, 1-β-D-arabinofuranosyl-, hydrochloride; cytarabine hydrochloride
Ascorbic acid: NSC-33832
BIC: NSC-82196; imidazole-4-carboxamide, 5-[3,3-bis(2-chloroethyl)nitrosoamino]carbonyl]amino]-2-deoxy-
BCNU: NSC-409962; urea, 1,3-bis(2-chloroethyl)-1-nitroso-; carmustine
Chitosan: NSC-127439
Chlorozotocin: NSC-178248; [1-D-glucopyranose, 2-[[[(2-chloroethyl)nitrosoamino]carbonyl]amino]-2-deoxy
Coenzyme Q_{10}: NSC-140865; *p*-benzoquinone, 2-(3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaenyl)-5,6-dimethoxy-3-methyl-
Concanavalin A: NSC-143504
Cycloheximide: NSC-185; glutarimide, 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]-
Cyclophosphamide: NSC-26271; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate
DDUG: NSC-109555; carbanilide, 4,4'-diacetyl-, bis(amidinohydrazone)-, dimethanesulfonate
Deoxyglucose: NSC-15193; D-arabinohexose, 2-deoxy-
Emetine: NSC-33669; 2*H*-benzo[*a*]quinolizine, 3-ethyl-1,3,4,6,7,11b-hexahydro-9,10-dimethoxy-2-[(1,2,3,4-tetrahydro-6,7-dimethoxy-1-isoquinolyl)methyl]-, dihydrochloride
5-FUdR: NSC-27640; uridine, 2'-deoxy-5-fluoro-
Hexamethylmelamine: NSC-13875; *s*-triazine, 2,4,6-tris(dimethylamino)-
Hydroxypropylcellulose ether: NSC-125428; klucel
Hydroxyurea: NSC-32065
ICRF-159: NSC-129943; 2,6-piperazinedione, 4,4'-propylenedi-, (±)-
Iminodipropanoldimethylsulfonate: NSC-102627; 1-propanol, 3,3'-iminodi-, dimethanesulfonate (ester), hydrochloride
Macromycin: NSC-170105
Mechlorethamine: NSC-762; diethylamine, 2,2'-dichloro-*N*-methyl, hydrochloride; nitrogen mustard
Melphalan: NSC-8806; alanine, 3-[*p*-[bis(2-chloroethyl)-amino]phenyl]hydrochloride, L-
MER-BCG: NSC-143769; methanol extraction residue of BCG
Methyl-CCNU: NSC-95441; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-, *trans*-
Methyl-GAG: NSC-32946; guanidine, 1,1-[(methylethanediylidene)dinitrilo]di-, dihydrochloride, monohydrate
Mitomycin C: NSC-26980; azirino[2',3':3,4]pyrrolo[1,2-*a*]indole-4,7-dione, 6-amino-1,1*a*,2,8,8*a*,8*b*-hexahydro-8-(hydroxymethyl)-8*a*-methoxy-5-methyl-, carbamate (ester)
Oxidized alginic acid: NSC-212577; poly[oxy(carboxymethylene)oxy(1,2-dicarboxy-1,2-ethanediyl)]; molecular weight about 1×10^5
Polyerga: NSC-165015
Puromycin: NSC-3055; adenosine, 3'-(α-amino-*p*-methoxyhydrocinnamamido)-3'-deoxy-*N*,*N*-dimethyl-, dihydrochloride
Pyrazole carboxamide: NSC-1402; pyrazole-4-carboxamide, 3(or 5)-amino-
Retinoic acid: NSC-122758; retinoic acid, all-*trans*
Rifampin: NSC-143414; rifampicin
Rose bengal: NSC-45732; fluorescein, 3,4,5,6-tetrachloro-2',4',5',7'-tetraiodo-, potassium deriv., potassium salt
Solanocapsine: NSC-152144; solanocapsine, dihydrochloride, hydrate
Sparsomycin: NSC-59729; 2-propenamide, *N*-[1-(hydroxymethyl)-2-[[(methylthio)methyl]sulfinyl]ethyl]-3-(1,2,3,4-tetrahydro-6-methyl-2,4-dioxo-5-pyrimidinyl)-, [*S*-(*E*)]-
Tetramisole: NSC-102063; imidazo[2,1-*b*]thiazole, 2,3,5,6-tetrahydro-*dl*-6-phenyl-, hydrochloride
Triethylenemelamine: NSC-9706; *s*-triazine, 2,4,6-tris(1-aziridinyl)-
Vincristine: NSC-67574; leurocristine sulfate (1:1)
Virazole: NSC-163039; 1*H*-1,2,4-triazole-3-carboxamide, 1-α-D-ribofuranosyl-
Zymosan: NSC-174798

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Pharmacologic Disposition of DL-Alanosine in Mice, Rats, Dogs, and Monkeys [1,2]

Janice M. Kelley, Richard H. Adamson,* David A. Cooney, Hiremagalur N. Jayaram, and Spurgeon Anandaraj [3,4]

SUMMARY

The pharmacologic disposition of DL-alanosine was studied in mice, rats, dogs, and monkeys. The major portion of the parenterally administered drug was excreted in the urine by all of the species studied; however, in rodents an important fraction of the dose was expired as CO_2. Organs in which DL-alanosine accumulates include the kidneys, lungs, liver, and small intestine. Considerable drug-derived radioactivity persists for periods up to 14 days in the hepatic and renal parenchyma. Studies on the clearance of DL-alanosine in mice using a specific enzymatic assay procedure indicated that the plasma half-life of the parent drug was shorter than the half-life of total radioactivity. In both cases, however, the kinetics were complex and not readily resolvable into discrete phases. In murine lymphoblasts (L5178Y/AR) the transport of DL-alanosine was found to be a sodium-stimulated, saturable, and thermosensitive process which was inhibited by L-threonine, L-asparagine, L-glutamine, L-serine, L-leucine, L-isoleucine, L-valine, L-cysteine, and L-homoserine. Kinetic studies on the influence of L-asparagine and L-glutamine on this transport revealed that the inhibition was competitive in nature.

[Cancer Treat Rep 61:1471–1484, 1977]

DL-Alanosine is a novel antibiotic produced by *Streptomyces alanosinicus* nov. sp. (ATCC-15710) (1). In addition to its antibacterial, antifungal, and antiviral properties, the antitumor activity of DL-alanosine has been demonstrated against a transplantable fibrosarcoma induced by SV-40 virus in hamsters (2) and against several experimental tumors in mice, the most notable being L1210 leukemia (1). Gale and Schmidt (3) first examined the mechanism of action of DL-alanosine and reported that the drug interferes with the conversion of inosine monophosphate to adenylosuccinate, a reaction requiring L-aspartic acid. On this basis and because L-aspartic acid counteracts the antibiotic effects of DL-alanosine versus *Candida albicans*, they classified the compound as an antagonist of L-aspartic acid. The present studies were designed to examine the fate and distribution of this novel antitumor antibiotic in several animal species prior to its use in man.

MATERIALS AND METHODS

DL-[1-^{14}C]alanosine (specific activities, 7.1–9.06 millicuries/mmol) (fig 1) was supplied by the Stanford Research Institute, Menlo Park, Calif. The purity of the radiochemical was > 95% as determined by thin-layer chromatography on MN cellulose and silica gel G plates in the following systems: (*a*) *n*-butanol saturated with 2 N HCl and (*b*) *N*-butanol, acetic acid, and water (85 + 55 + 55 ml). When greater purity was required, as for example in binding studies, the drug was adsorbed onto DEAE sephadex in the formate form and eluted with a 0.05–1.0 M gradient of tris-formate, pH 7.4. In some cases, nonradioactive DL-alanosine, synthesized by

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[2]DL-Alanosine: NSC-143647; CAS reg. No. 5854-95-5; propionic acid, 2-amino-3-(hydroxynitrosamino)-, DL-.

[3]Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

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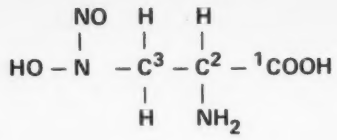
**Reprint requests to:* Dr. Richard H. Adamson, DCT, NCI, Bldg 37, Rm 5A17, National Institutes of Health, Bethesda, Md 20014.



DL – ALANOSINE

DL – [Propionic acid, 2 – amino – 3 – (hydroxynitrosamino)]

FIGURE 1.—Structure and chemical designation of DL-alanosine.

the Dow Chemical Company, Indianapolis, Ind, and supplied by the Division of Cancer Treatment, National Cancer Institute (NCI), was added to labeled drug. The mixture was dissolved in saline or 1% sodium bicarbonate ($NaHCO_3$) and administered at the doses indicated via the ip, iv, oral, or sc routes.

One hundred CDF_1 or 20 BDF_1 male mice (20–25 g), 30 male Sprague-Dawley rats (250–300 g), six mongrel dogs (7–11 kg), and five female rhesus monkeys (6–8 kg) were used in this study. Unless otherwise specified, the doses injected were as follows: mice and rats, 20 mg/kg (in a maximum volume of 0.01 ml/g of body weight); and dogs and monkeys, 10 mg/kg (in a maximum volume of 10.0 ml administered within 60 seconds). The routes of administration were oral or sc to CDF_1 mice, iv to BDF_1 mice, ip or iv to Sprague-Dawley rats, and iv to mongrel dogs and monkeys. Facilitation of iv injections in the dogs and monkeys was accomplished by cannulation of the femoral vein and artery with polyethylene tubing.

Urine and blood samples were collected at varying time intervals as indicated in the appropriate tables. Mice and rats were housed in glass or polycarbonate metabolism cages (4) and provided water ad libitum for the duration of the study. Mice were killed by cervical dislocation following blood collection from the ophthalmic or axillary vessels; the bladder was excised and any residual urine removed with a tapered pipette. Urine was collected from dogs and monkeys by means of a pediatric Foley catheter for female animals and an adult urethral catheter for male animals.

Blood samples were collected in heparinized tubes and centrifuged at 2000 *g* for 10 minutes. Radioactivity of plasma was assessed by counting 0.01–0.1 ml in the Packard automatic Tri-Carb liquid scintillation spectrometer (model 4322) or the Beckman LS230 liquid scintillation spectrometer using a fluor consisting of 1,4-bis-2(5-phenyloxazolyl)-benzene and 2,5-diphenyloxazole dissolved in a binary mixture of methanol or ethanol and toluene (1:2, vol/vol) at 85% efficiency. The total volume of urine samples was recorded and 0.1-ml aliquots were analyzed for radioactivity by counting directly in the toluene fluor previously mentioned.

Bile from the rats and cerebrospinal fluid (CSF) samples from the dogs and monkeys were collected by cannulation of the common bile duct or cisterna magna, with PE 50 and 100 tubing respectively. Bile samples were treated with 0.5 ml of NCS (Amersham/Searle Corp, Arlington Heights, Ill) tissue solubilizer/0.1 ml of sample and dispersed in 18 ml of the toluene fluor, and the radioactivity was measured in the Packard spectrometer. Samples of CSF were counted directly in the toluene fluor. In all cases, appropriate quench corrections were made.

For fecal excretion studies, mice and rats were fitted with anal cups; the total excreta were weighed and homogenized and an aliquot was treated with NCS at 40°C for 12–24 hours; subsequent measurements of radioactivity were carried out as described earlier.

For the protein-binding studies, either mouse plasma or 1% (weight/vol) bovine serum albumin (fraction 5) was prepared in 0.05 M tris-HCl buffer, pH 8.4. Ten-μl aliquots of chromatographically purified DL-[1-^{14}C]alanosine in amounts ranging from 13 to 26 nmols were added to 1 ml of this solution. One ml of the resultant solution was pipeted into prewashed sacs prepared from cellulose tubing 8 mm in diameter, then dialyzed against an equal volume of buffer at 4°C for 16 hours, whereafter the radioactivity of aliquots from the inner and outer compartments was measured by scintillation spectrometry.

For studies on the disposition of DL-alanosine, the mice were injected with radioactive DL-alanosine (the route of administration and the amount injected are specified in the appropriate legends) and their tissues were removed at the times indicated, frozen on dry ice, and weighed. Using the Sorvall-Omni mixer or the Waring blender, tissues

and residual carcasses were separately homogenized in a known volume of 0.05 M tris-HCl buffer, pH 8.4; aliquots of the homogenates were treated with NCS, as previously described. Twenty ml of scintillation fluid was added and the digests were counted. Chromatographic analyses of the high-speed supernatants (100,500 *g* for 1 hour) of selected organ homogenates prepared in 0.05 M tris-HCl buffer, pH 8.4, and dialyzed overnight at 4°C against a large excess of the homogenization buffer were carried out on DEAE sephadex A-25, formate form, equilibrated with 0.01 M tris-formate, pH 7.0, using a 0.1–1.0 M ammonium formate, pH 7.0, gradient.

The amount of radioactivity in expired CO_2 was determined by individually placing 12 CDF_1 mice (eight normal and four gut sterilized by treatment with neomycin [4 mg/ml] and sulfathiazole [1 mg/ml] in drinking water for 7 days [4]) or three Sprague-Dawley rats in respiration chambers; CO_2 was collected for 24 hours in 1 N NaOH (5). A 3-ml aliquot of this NaOH was mixed with 1 g of anthracene (scintillation grade) containing Triton X-100 and then counted.

In vitro incubations of various tissues from CDF_1 male mice with DL-[1-^{14}C]alanosine were carried out as follows: 1 g of tissue-mince or 250 mg of the appropriate subcellular fraction (homogenate, 9000 *g* supernatant, mitochondria, or microsomes), 2.0 ml of Locke's solution, 1.8 ml of 0.1 M tris-HCl buffer, pH 7.4, and 0.2 ml of DL-[1-^{14}C]alanosine (400 μg) were added to stoppered incubation flasks with center wells containing 0.4 ml of 2 N KOH; incubations were carried out for 1 hour at 37°C. At the end of this time, 0.2 ml of 60% perchloric acid was added to the mixtures for the purpose of terminating the reaction; 1 hour later 0.1-ml aliquots of KOH were taken from the center wells for scintillation counting.

Measurement of Nonmetabolized (Unaltered) DL-[1-^{14}C]alanosine in Mouse Plasma With L-Glutamate Decarboxylase

At various times after the iv injection of 10 microcuries of DL-[1-^{14}C]alanosine into a caudal vein of adult BDF_1 mice, heparinized blood (~100 μl) was taken from the ophthalmic sinus and centrifuged immediately at 12,000 *g*. Ten-μl aliquots of the plasma were dispensed in triplicate into Eppendorf 1500-μl test tubes. The DL-[1-^{14}C]alanosine in the samples was then decarboxylated by the addition of 1 IU of *Escherichia coli* L-glutamate decarboxylase ([EC 4.1.1.15] purified by the method of Shukuya and Schwert [6]) in 50 μl of 0.66 M sodium acetate buffer, pH 4.2; any $[^{14}C]O_2$ generated in this way was trapped in 5-μl droplets of 40% KOH deposited on the underside of the lids of the vessels. After 6 hours at 37°C, the lids were removed and the trapped radioactivity was quantitated by scintillation spectrometry. Recoveries by this technique ranged from 30% to 45% of the radioactivity of standard samples of DL-[1-^{14}C]alanosine or from 60% to 90% of the concentration of the L-isomer present in the racemic mixture. The product of these incubations remained at the origin, ie, it was neutral on high-voltage paper electrophoresis conducted in 0.1 M phosphate buffer, pH 7.0, at 2000 V, 100 mA, for 1 hour. Under the same experimental conditions no alteration in the electrophoretic behavior of the following analogs of alanosine was produced by exposure to L-glutamate decarboxylase: D-alanosine, propionic acid, 2-amino-3-(hydroxynitrosamino); propionic acid, 2-oxo-3-(hydroxynitrosamino); ethylamine, 2-(hydroxynitrosamino); propionic acid, 2-hydroxy-3-(hydroxynitrosamino); and propionic acid, 2-ureido-3-(hydroxynitrosamino).

Cellular Uptake Studies

One million cells of L5178Y/AR leukemia (L5178Y rendered resistant to L-asparaginase by repeated subcurative doses of the enzyme) were injected ip into BDF_1 mice. After 7–9 days, ascitic fluid was aspirated from the peritoneum of the tumor-bearing mice and the cellular pellet was washed twice with Hank's balanced salt solution (HBSS) containing 25 mM HEPES. The lymphoblasts were then suspended in the same buffer to achieve a final concentration of 5×10^6 cells/ml and equilibrated at 37°C in an atmosphere of 95% air and 5% CO_2.

Aliquots (500 μl) of the above cell suspension were dispensed into Eppendorf test tubes at 37°C. At time 0, 5 μl of a 0.1 M solution of amino acid as shown in the appropriate table (final concentration of 1 mM) was added and mixed by inversion; 1 minute later, 1.4 microcuries of DL-[1-^{14}C]alanosine (specific radioactivity, 7.14 millicuries/mmol) was added and mixed. Every 15 seconds thereafter a 50-μl aliquot was sampled and immediately dispensed into an Eppendorf test tube containing 1 ml of ice-cold HBSS; the contents were mixed and immediately centrifuged for one half minute at 12,000 *g* in the Eppendorf centrifuge. Thereafter, the cells were rapidly washed twice with 1 ml of ice-cold HBSS. Twenty μl of 40% KOH was added to the washed cell pellet and the cells were digested at 95°C for 20 minutes. The entire vessel was then immersed in scintillation fluid and counted.

Studies on the influence of temperature on the uptake of DL-alanosine were carried out in an

analogous way except that the solutions used were preadjusted to temperatures ranging from 4°C to 37°C for 5 minutes prior to the addition of DL-alanosine.

Studies of the sodium-dependence of DL-alanosine uptake followed the protocols given above, except that 10 mM tris-HCl containing 15 mM $MgCl_2$ and 25 mM HEPES replaced HBSS as the basic medium; sodium concentrations in the modified medium ranged from 0 to 150 mM (7).

For kinetic studies on the transport of DL-alanosine in the presence and absence of metabolic inhibitors, the concentration of the antibiotic in the medium (HBSS) ranged from 0.001 to 0.082 M; in some cases sodium cyanide (0.5–50 mM) or 2,4-dinitrophenol (5–50 mM) was also included.

For kinetic studies on the inhibitory characteristics of L-asparagine and L-glutamine on the transport of DL-alanosine by L5178Y/AR cells, the same procedure for the preparation of cells was used as described earlier. Aliquots (100 ml) of the cell suspension in HBSS (1×10^8/ml) were dispensed into Eppendorf test tubes at 37°C. The natural amino acids (final concentration, 4 or 8 mM) and DL-[1-^{14}C]alanosine (1.4 microcuries, specific radioactivity, 7.14 millicuries/mmol) were added to the cells simultaneously. After 1 minute, 1 ml of 1 mM L-alanosine in ice-cold HBSS was injected through the lid, and the vessels were immediately centrifuged for one-half minute at 12,000 *g*; thereafter the cell pellet was processed as described earlier.

RESULTS

Studies in Mice

In preparation for the studies of the disposition of DL-alanosine in vivo, the ability of the drug to bind to crude mouse plasma proteins and also to purified bovine serum albumin was examined in vitro. As judged by equilibrium dialysis, < 1% of the antibiotic became affiliated with these macromolecules. This finding excluded protein binding as a significant factor in the clearance of DL-alanosine.

The plasma clearance of radioactivity after an ip administration of DL-[1-^{14}C]alanosine into CDF_1 mice was biphasic, with an initial half-life of approximately 40 minutes and a more prolonged half-life of approximately 2 hours. As measured by a specific enzymatic technique, the kinetics of removal of parent or unchanged DL-alanosine from plasma of BDF_1 mice after iv administration were even more complex, with at least three phases discernible on the semilog plot (fig 2). Most noteworthy is the observation that the fraction of nonmetabolized DL-alanosine in plasma decreased within 80 minutes to negligible levels.

Table 1 summarizes the urinary excretion of radioactivity by mice following ip, iv, sc, or oral administration of 20 mg/kg of DL-[1-^{14}C]alanosine. The pattern of excretion of radioactivity was similar following administration by all routes; total urinary excretion at 48 hours appeared to be 65% of the

TABLE 1.—Excretion of DL-alanosine by mice (cumulative % of administered dose)*

Route of administration	Time interval (hrs)	% urine average (range)	% feces average	% total
Ip	1	17 (13–23)		17
	4	27 (23–31)		27
	24	44 (34–55)	0.4	44
	48	45 (36–55)	1.3	46
Iv	1	18 (12–25)		18
	4	37 (33–43)		37
	24	55 (55–56)	0.5	56
	48	65 (64–66)	0.7	66
Oral	1	7 (2–11)		7
	4	29 (20–38)		29
	24	46 (40–57)	0.7	47
	48	54 (43–66)	0.9	55
Sc	1	10 (5–13)		10
	4	32 (28–37)		32
	24	51 (48–56)	0.5	52
	48	62 (58–65)	0.6	63

*Groups of 5 CDF_1 mice were injected by the routes indicated with 20 mg/kg of DL-alanosine containing 1.5 microcuries of DL-[1-^{14}C]alanosine. After specified time intervals, the urine and feces were collected and analyzed for radioactivity as described in Materials and Methods. The results are expressed as cumulative % of administered dose.

TABLE 2.—Distribution of radioactivity at 24 and 48 hrs after administration of DL-alanosine to BDF_1 mice*

Tissue	Distribution (mean ± SE) (24 hrs)		Distribution (mean ± SE) (48 hrs)	
	% of administered dose	dpm × 10^3/g	% of administered dose	dpm × 10^3/g
Brain	0.14 ± 0.02	11 ± 0.1	0.12 ± 0.02	15 ± 1.0
Gonads	0.20 ± 0.05	27 ± 6.0	0.2 ± 0.06	22 ± 2.0
Heart	0.10 ± 0.00	15 ± 4.0	0.1 ± 0.00	21 ± 3.0
Kidneys	1.20 ± 0.02	91 ± 12.0	0.7 ± 0.10	74 ± 11.0
Large intestine (with contents)	1.30 ± 0.20	60 ± 11.0	0.7 ± 0.10	46 ± 5.0
Liver	5.30 ± 0.80	161 ± 57.0	3.1 ± 0.60	96 ± 12.0
Lungs	1.00 ± 0.10	115 ± 16.0	0.5 ± 0.05	124 ± 5.0
Seminal vesicle	0.30 ± 0.08	46 ± 11.0	0.2 ± 0.04	34 ± 4.0
Small intestine (with contents)	2.60 ± 0.20	52 ± 8.0	2.1 ± 0.30	54 ± 9.0
Spleen	0.20 ± 0.03	82 ± 9.0	0.1 ± 0.02	73 ± 10.0
Stomach	0.50 ± 0.04	73 ± 6.0	0.4 ± 0.04	64 ± 3.0
Residual carcass	18.30 ± 1.30		12.2 ± 0.60	
Feces	0.30 ± 0.06		1.25 ± 0.10	
Urine	42.00 ± 4.00		47.80 ± 0.40	
Tumor†	0.11 ± 0.035	57 ± 22.0	0.13 ± 0.05	22 ± 9.0
Totals	79.08 ± 5.50		69.55 ± 3.30	

*Groups of 8 BDF_1 mice were injected ip with a dose of 20 mg/kg of DL-alanosine containing 1.5 microcuries of DL-[1-^{14}C]alanosine; at 24 and 48 hrs tissues were excised and processed as described in Materials and Methods.

†Male BDF_1 mice, weighing between 18 and 20 g, were injected sc with a suspension of 10^6 cells of L5178Y leukemia made resistant to L-asparaginase by subcurative doses of the enzyme. 7–10 days later, tumor-bearing mice were injected ip with 20 mg/kg of DL-alanosine containing 1.5 microcuries of DL-[1-^{14}C]alanosine; 24 and 48 hrs later, liver, kidneys, and tumor nodule were excised from groups of 4 mice and processed as detailed in Materials and Methods. The distribution profile of radioactivity in liver and kidneys of these tumor-bearing mice at 24 hrs was 3.0% ± 0.69% and 1.0% ± 0.5% and at 48 hrs was 3.3% ± 0.83% and 0.32% ± 0.14% respectively.

dose following iv or sc administration and approximately 50% of the dose following ip or oral administration. It is important to note that absorption of DL-alanosine was significant even after oral administration. In addition, irrespective of the route of administration, only ≤ 1% of the radioactivity was found in the feces.

The distribution of radioactivity in organs, tissues, urine, and feces of mice at 24 and 48 hours following an ip injection of DL-[1-^{14}C]alanosine into normal mice and mice bearing L5178Y/AR tumor is summarized in table 2. The distribution of radioactivity was comparable in both normal and tumor-bearing mice and the highest percentage of radioactivity at 24 hours was found in the urine, liver, kidneys, lungs, large and small intestines, and carcass. Only 0.1% of the total radioactivity was recovered in the subcutaneous tumor. When expressed as dpm/g of tissue, the liver and lungs were identified as organs with the greatest concentration of the label. At 48 hours, the greatest percentage of radioactivity was again found in the liver, small intestine, and carcass; on a concentration basis, the lungs and liver retained substantial radioactivity. At 24 or 48 hours, the total radioactivity accounted for 70%–80% of the administered dose.

It is noteworthy that 5 days after a single iv injection of DL-[1-^{14}C]alanosine into BDF_1 mice, significant levels of radioactivity persisted in the principal viscera; even after 14 days, the liver and kidneys contained considerable amounts of label (fig 3). This persistent radioactivity appeared to decline with an approximate half-life of 5–14 days. DEAE sephadex chromatography of the soluble radioactive macromolecules present in the liver 5 days after an iv injection of DL-[1-^{14}C]alanosine revealed that the majority of the label was distributed among compounds eluting early (peptides and proteins) as opposed to those compounds eluting later (nucleic acids).

The fact that approximately 20% of the injected label in the plasma clearance studies was not accounted for prompted us to examine the expiration of [^{14}C]O_2 by normal and gut-sterilized mice following the administration of DL-[1-^{14}C]alanosine. The amount of [^{14}C]O_2 found in the expired air 24 hours after administration of DL-[1-^{14}C]alanosine was 17.69% ± 1.83%. A similar percentage of radioactivity was found in the expired air of gut-sterilized mice (17.86% ± 1.03%), suggesting that enteric bacteria did not metabolize the drug to CO_2.

Evidence that the liver is an important site of DL-alanosine metabolism was provided by the studies presented in table 3, where it can be seen that 1% of the drug was degraded to CO_2 in vitro per hour per g of tissue under the conditions studied.

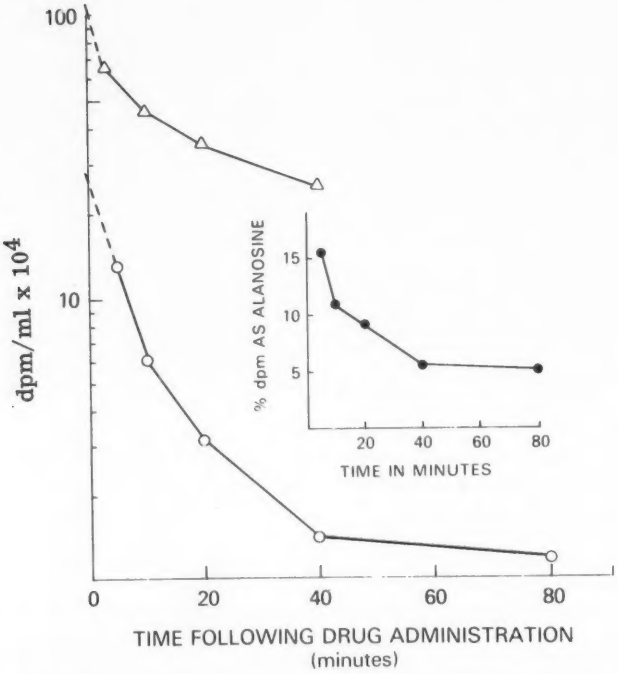


FIGURE 2.—Clearance of DL-[1-^{14}C]alanosine from plasma following iv injection. Groups of 5 mice were given iv injections of 10 microcuries of DL-[1-^{14}C]alanosine and were bled individually from the ophthalmic sinus at the times indicated. Blood was centrifuged immediately at 12,000 *g*, and total radioactivity in the plasma (△) and radioactivity as DL-[1-^{14}C]alanosine (○) were measured by the methodology given in Materials and Methods. Insert in the figure indicates the % clearance of radioactivity as DL-alanosine measured by the enzymatic methodology detailed in Materials and Methods.

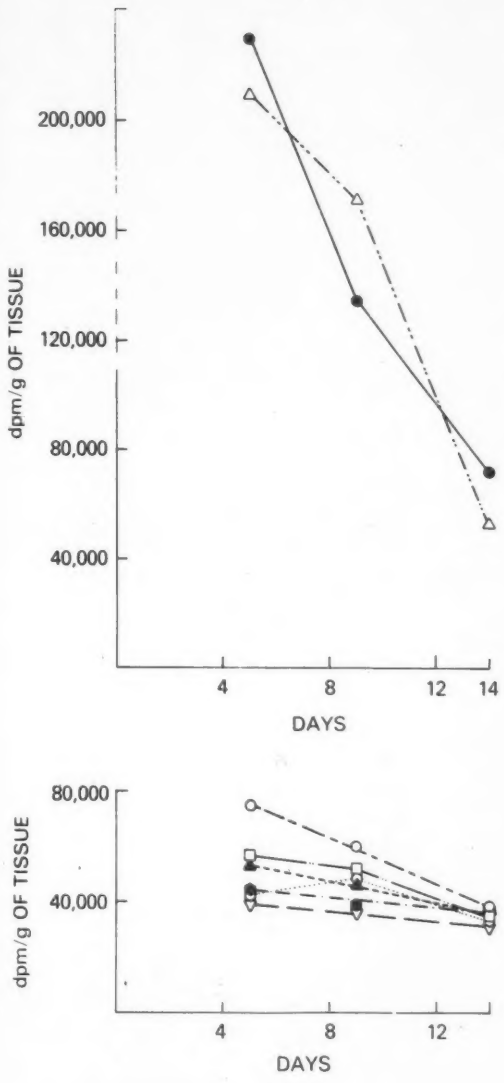


FIGURE 3.—Decay of radioactivity derived from DL-[1-^{14}C]alanosine in selected organs of the mouse. Groups of 5 BDF$_1$ mice received 10 microcuries of DL-[1-^{14}C]alanosine on Day 0. At the times indicated, tissues were excised and prepared for scintillation counting as described in Materials and Methods. ● = liver, △ = kidneys, ○ = lungs, □ = carcass, ▲ = pancreas, ⬢ = brain, ⬡ = spleen, ▽ = small intestine.

When various hepatic cell fractions were examined for their ability to metabolize DL-alanosine to CO_2, it was found that (other than whole homogenate) the mitochondrial fraction and the 9000-*g* supernatant exhibited the highest activity, with approximately 0.5% of the dose recovered per g per hour as [^{14}C]O_2 from these fractions. Furthermore, hydrazine sulfate was found to interfere with the metabolism of DL-alanosine to CO_2, the inhibition by this pyridoxal-reagent being 62%, 39%, and 31% at concentrations of 1.0, 0.1, and 0.01 mM respectively.

Since the data presented in table 2 indicated that DL-alanosine gains entry into several mouse organs at a rapid rate, experiments were designed to examine the transport mechanism by which the antibiotic penetrates single cells. Murine lymphoblasts (L5178Y/AR) were chosen for these studies in view of the convenience with which monocellular suspensions could be prepared; HBSS was chosen as the medium since it is free of other amino acids that are likely to compete with DL-alanosine for entry. In this system, the rate of transport of DL-[1-^{14}C]alanosine was 1.07 nmols/million cells/minute at 37°C, as contrasted with 0.06 nmol/million cells/minute at 4°C (fig 4a). This finding suggests that the principal mechanism of entry of DL-alanosine into these cells is by an active transport process. Support for the contention was provided by the finding that the uptake of DL-alanosine was saturable (fig 5): the apparent affinity of the transporting mechanism for DL-alanosine, Km apparent, is about 16 mM. In addition, sodium ions were found to stimulate the uptake of DL-alanosine to a prominent degree. Thus, the uptake of drug in a sodium-free system at 37°C was 0.45 ± 0.08 nmol/million cells/minute; 10 mM NaCl elevated this transport to 0.65 ± 0.07 nmol/million cells/minute and 150 mM NaCl more than doubled the basic transport to 1.19 ± 0.04 nmols/million cells/minute. Additional evidence for the active nature of the transport process was provided by the observation that 50 mM NaCN inhibited the uptake of DL-alanosine by 82%, 5 mM by 77%, and 0.5 mM by 45%.

Pursuant to the finding that DL-alanosine was transported actively into L5178Y/AR cells, attempts were made to define the transport group to which this fraudulent amino acid belonged. Towards this end, cells were exposed first to normal amino acids, and 1 minute later to DL-[1-^{14}C]alanosine. Of the dicarboxylic amino acids examined, L-asparagine and L-glutamine interrupted the uptake of DL-alanosine to a prominent degree at a concentration of 1 mM, whereas L-aspartic acid and L-glutamic acid did not. Most neutral amino acids (table 4) also inhibited the entry of DL-alanosine, whereas the

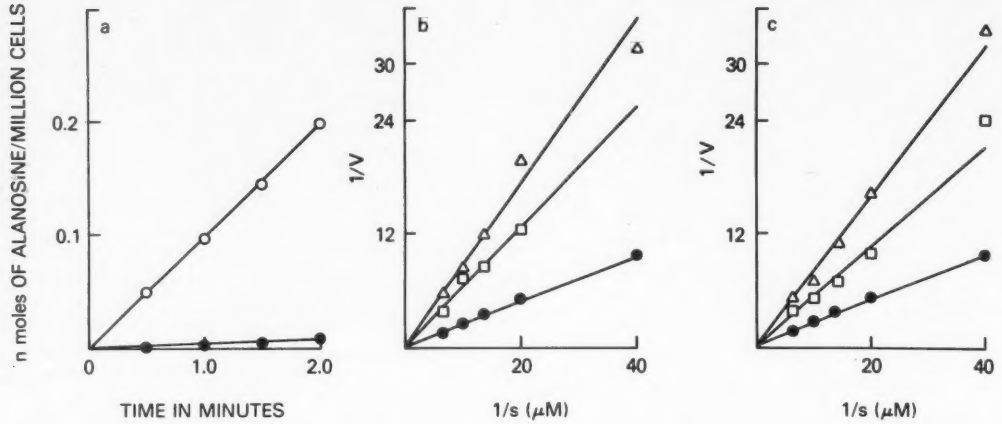


FIGURE 4.—Characteristics of the uptake of DL-[1-^{14}C]alanosine by isolated cells of L5178Y/AR leukemia. The procedure used for the uptake studies is detailed in Materials and Methods with the following modifications: (*a*) study of the time course of DL-[1-^{14}C]alanosine uptake was conducted at 37°C (○) and at 4°C (●); (*b*) measurement of the inhibition of the uptake of DL-[1-^{14}C]alanosine by L-asparagine was carried out at 4.0 mM (□) or 8.0 mM (△) L-asparagine and compared to the uptake in the absence of L-asparagine (●); and (*c*) measurement of the inhibition of the uptake of DL-[1-^{14}C]alanosine by L-glutamine was carried out at 4.0 mM (□) or 8.0 mM (△) L-glutamine and compared to the uptake in the absence of L-glutamine (●).

TABLE 3.—In vitro metabolism of DL-[1-^{14}C]alanosine to [^{14}C]O_2 by minced preparations of various mouse tissues*

Tissue	% of DL-[1-^{14}C]alanosine recovered as [^{14}C]O_2 (mean ± SE)
Kidneys	0.34 ± 0.05
Large intestine (with contents)	0.32 ± 0.07
Liver	1.18 ± 0.02
Lungs	0.15 ± 0.01
Small intestine (with contents)	0.34 ± 0.30
Blood	0.00

*Tissues were excised from 5 CDF_1 mice and minced (1-mm cubes) in 0.05 M tris-HCl buffer, pH 7.4 (1:4 wt/vol). 1 ml of minced suspension was transferred to scintillation vials. The reaction was started by adding 1 microcurie of DL-[1-^{14}C]alanosine and by capping immediately with polypropylene caps fused with a center well containing 100 μl of 40% KOH. After incubating for 1 hr at 37°C, the center wells were cut off and the radioactivity was measured as described in Materials and Methods.

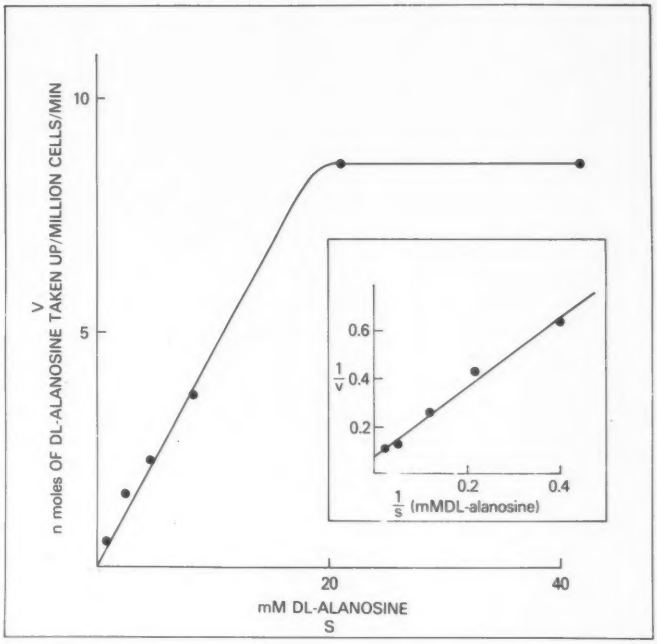


FIGURE 5.—Uptake of DL-alanosine by L5178Y/AR cells. Measurement of the uptake of DL-[1-^{14}C]alanosine (at concentrations ranging from 1 to 40 mM) by tumor cells was carried out according to the methodology described in Materials and Methods. Insert in the figure shows a plot of inverse concentration of DL-alanosine versus inverse velocity of uptake.

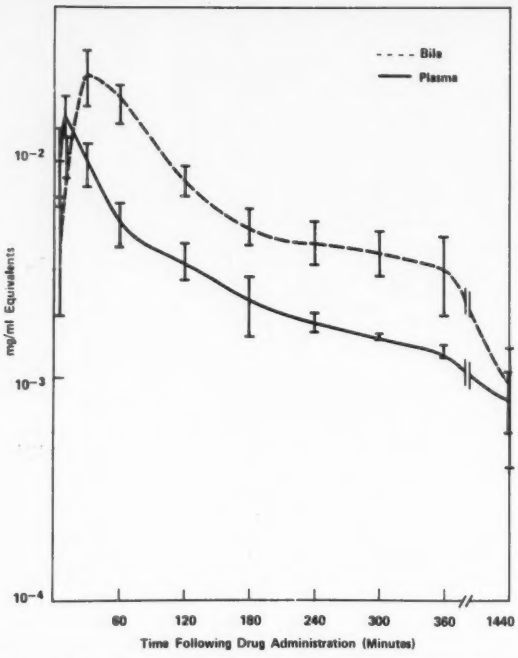


FIGURE 6.—Clearance of DL-alanosine in plasma and bile of rats injected ip with 20 mg/kg of DL-alanosine over a period of 24 hrs. Sprague-Dawley rats were injected ip with 20 mg/kg of DL-alanosine containing 1 microcurie of DL-[1-^{14}C]alanosine, and at the indicated time periods groups of 5 rats were killed, bile and plasma were collected, and an aliquot was counted as described in Materials and Methods.

basic and aromatic amino acids in general failed to do so. Whereas D-asparagine produced no inhibition of uptake, D-glutamine was equivalent to L-glutamine. Kinetic analysis of the inhibition exerted by L-asparagine and L-glutamine yielded patterns which were formally competitive in type, as shown in figures 4b and 4c.

Studies in Rats

Excretion of radioactivity by male rats following ip administration of 20 mg/kg of DL-[1-^{14}C]alanosine was studied next. One hour after administration, approximately 14% of the dose was found in the urine; renal excretion of radioactivity increased to 58% in 24 hours and to 62% in 48 hours. Again, as with the mice, $< 1\%$ of the dose appeared in the feces. The percentage of radioactivity found in the bile of rats with cannulated bile ducts was small, and the bile/plasma ratio was somewhat > 1 at all time intervals for the duration of the 24-hour experiment, but was never great enough to suggest a process other than passive diffusion (fig 6). Furthermore, only a small percentage of the dose was detected in the bile in 24 hours (1.2%). Experiments utilizing a metabolic chamber for CO_2 collection demonstrated that approximately 10% of the dose given to rats was excreted as $[^{14}C]O_2$ in the first 4 hours following injection of DL-alanosine; thus, the breakdown of DL-alanosine to CO_2 occurs fairly rapidly. When the mean percent of $[^{14}C]O_2$ expired by rats is added to the percent radioactivity recovered in the urine, feces, and tissues, a total of $> 92\%$ of the dose could be accounted for.

In rats, 24 hours after administration of DL-[1-^{14}C]alanosine, the principal sites of radioactivity were the liver, kidneys, lungs, gastrointestinal tract, and urine (table 5). Conversion of the data to dpm/g of tissue (not shown in the table) indicates that the kidneys contained the highest concentration of radioactivity followed by the lungs, liver, and gastrointestinal tract in that order.

Studies in Dogs

Two male and four female dogs were used to study the urinary excretion and plasma disappearance of DL-alanosine injected iv. Excretion averaged approximately 40% for the first 6 hours and approximately 55% for the first 24 hours. The percent cumulative dose excreted by male and female dogs was similar. The plasma disappearance of radioactivity and the appearance of radioactivity in the CSF of three dogs are summarized in figure 7. A biphasic plasma clearance was seen, with the initial half-life approximating 15–20 minutes and the second half-life being more prolonged (~180 minutes). Radioactivity in CSF was one third to one sixth of that found in plasma at comparable time periods.

Studies in Monkeys

The urinary excretion of DL-alanosine, the plasma disappearance of radioactivity, and the organ-distribution of radioactivity in monkeys are presented in table 6 and figure 8. Urinary excretion was fairly rapid, with 20%–36% of the dose excreted in 3 hours and 50%–60% excreted in 24 hours. Radioactivity remaining in the tissues and organs of monkeys was small; the liver retained the greatest percentage of radioactivity but this nevertheless amounted only to ~1% of the dose. Bile contained a minor amount of label. The total recovery of radioactivity from urine, feces, and organs examined was 54%–64%.

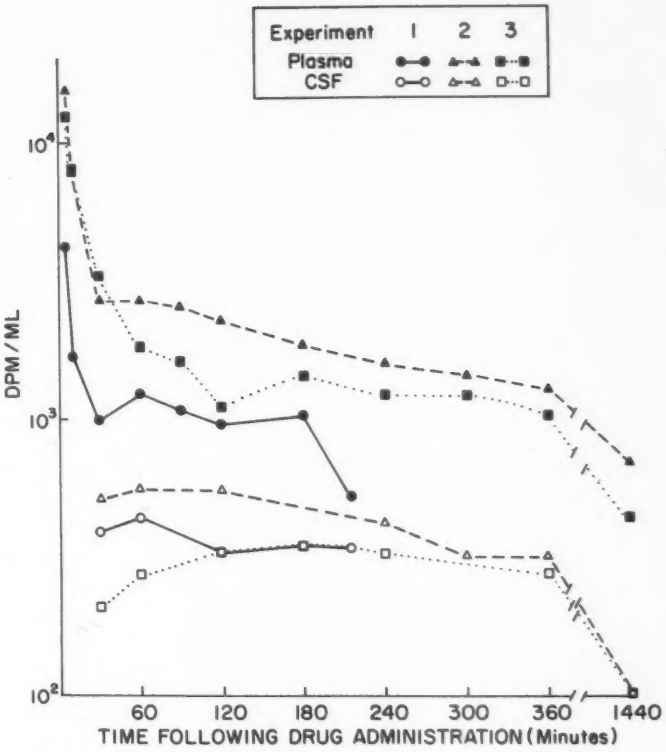


FIGURE 7.—Removal of DL-[1-^{14}C]alanosine from dog plasma and its penetration into CSF at various times after iv injection of the drug. Procedural details are given in Materials and Methods.

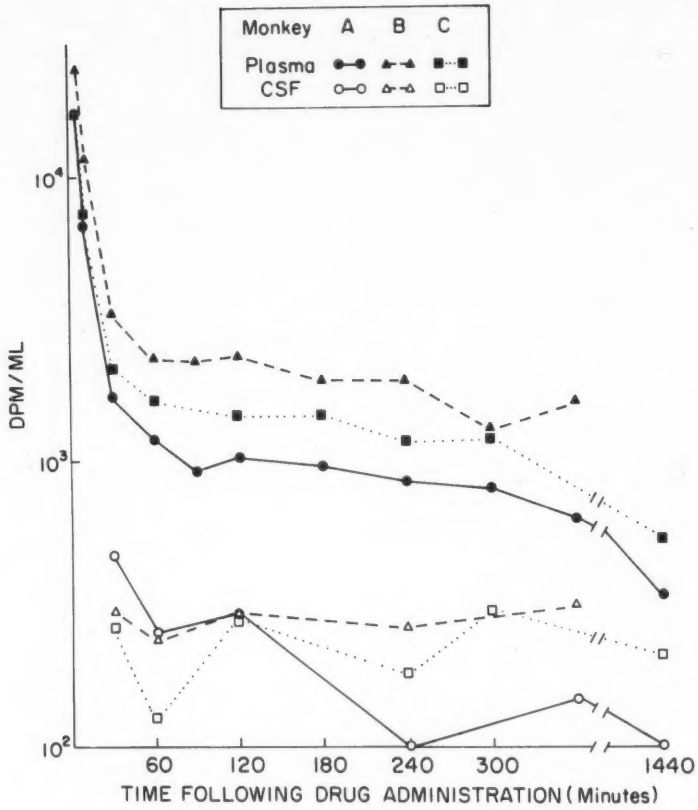


FIGURE 8.—Removal of DL-[1-^{14}C]alanosine from monkey plasma and its penetration into CSF at various times after iv injection of the drug. Procedural details are given in Materials and Methods.

TABLE 4.—Effect of various amino acids on the uptake of DL-[1-^{14}C]alanosine by murine lymphoblasts (L5178Y/AR cells)*

Amino acids	% inhibition
L-Threonine	50
L-Asparagine	46
L-Serine	40
L-Glutamine	38
L-Cysteine	37
L-Homoserine	37
L-Isoleucine	35
L-Valine	31
L-Leucine	30
Glycine	29
L-Methionine	25
L-Alanine	18
D-Glutamine	39
D-Asparagine	0

*Cells were prepared and the effects of various amino acids (final concentration of 1.0 mM) were studied by the methodology detailed in Materials and Methods. The uptake of DL-[1-^{14}C]alanosine is expressed as nmols of DL-alanosine taken up by 1 million cells/min at 37°C. In the controls, where no amino acids were added, 1.07 nmols of DL-[1-^{14}C]alanosine was taken up by 1 million cells/min at 37°C. L-Cystine, L-aspartic acid, L-glutamic acid, L-arginine, L-histidine, L-proline, L-lysine, L-tryptophane, L-phenylalanine, and L-tyrosine showed negligible effects on the uptake of DL-[1-^{14}C]alanosine under the conditions of this assay.

TABLE 5.—Distribution of radioactivity in rats 24 hrs after ip administration of DL-[1-^{14}C]alanosine*

Tissue	% of dose
Plasma	0.10
Bladder	0.04
Brain	0.05
Gonads	0.52
Heart	0.10
Kidneys	1.15
Large intestine (with contents)	0.27
Small intestine (with contents)	0.27
Liver	2.36
Lungs	2.06
Seminal vesicles	0.08
Spleen	0.31
Stomach	0.65
Muscle	0.03
Residual carcass	16.92
Feces	0.50
Urine	55.49
CO_2	11.00
Total recovery	91.90

*8 male Sprague-Dawley rats were injected ip with 20 mg/kg of DL-[1-^{14}C]alanosine containing 1.5 millicuries of DL-[1-^{14}C]alanosine, then transferred to metabolism cages. 24 hrs after the administration of the drug, various tissues were excised and processed as described in Materials and Methods.

TABLE 6.—Distribution of radioactivity in monkeys at 24 hrs after iv administration of DL-[1-^{14}C]alanosine*

Tissue	% of dose	dpm/g
Kidneys	0.34 (± 0.15)	4996
Liver	0.78 (± 0.14)	2309
Lungs	0.20 (± 0.08)	1622
Spleen	0.03 (± 0.01)	1567
Bile	0.003 (± 0.001)	55
Urine	57.45 (± 5.05)	
Feces	0.50 (± 0.10)	
Total	59.04	

*DL-Alanosine (20 mg/kg, containing 20 microcuries of DL-[1-^{14}C]alanosine) was administered iv to female rhesus monkeys. Tissues were excised 24 hrs after the administration of the drug and processed as described in Materials and Methods.

The plasma clearance in the monkeys was biphasic, with an initial half-life of approximately 8 minutes followed by a prolonged second half-life (~300 minutes). Radioactivity in CSF was approximately one fourth to one tenth of that found in plasma at comparable collection periods.

DISCUSSION

DL-alanosine has antitumor activity in several experimental tumor systems including L1210 leukemia (1); the L-isomer is also a therapeutic agent effective against L1210 leukemia. Studies on the disposition of DL-[1-^{14}C]alanosine (fig 1) in mice revealed that the compound is rapidly removed from plasma and is readily excreted in the urine, regardless of the route of administration. In addition, the mouse metabolizes DL-alanosine extensively with approximately 18% of the dose being expired as [^{14}C]O_2; this percentage did not change in mice gut-sterilized with neomycin sulfate and sulfathiazole. In vitro incubations of DL-alanosine with various tissues revealed the liver to be the major organ involved in the metabolism of the drug. Studies on tissue distribution of radioactivity at 24 hours in mice documented that the lungs, liver, and kidneys contained the greatest amount of radioactivity, presumably because they are involved in the excretion and metabolism of DL-alanosine.

Studies on urinary excretion and expired CO_2 in the rat revealed that approximately 60% of the dose administered was recovered in 24 hours and an additional 10% was recovered as [^{14}C]O_2. Studies on biliary excretion in the rat revealed that the bile/plasma ratio was slightly > 1.0 at all time intervals after 5 minutes but nevertheless only a

small percentage of the total radioactivity was excreted in the bile.

Studies on the disposition of DL-alanosine in the dogs and monkeys revealed a biphasic plasma half-life, with an initial rapid half-life (8–15 minutes) followed by a slower decline (180–300 minutes). Urinary excretion studies in the dogs and monkeys documented that 40%–70% of the administered dose was recovered within 24 hours following an iv administration of DL-alanosine. In monkeys, as in rodents, < 1% of the dose was found in the feces. On the other hand, samples of CSF in the dogs and monkeys demonstrated that the drug penetrated this compartment to an appreciable extent, reaching levels approximately one fifth that of plasma at comparable time periods.

Several instances of published evidence indicate that DL-alanosine is an analog of the dicarboxylic amino acids: thus, the drug has been shown to inhibit adenylosuccinate synthetase (EC 6.3.4.4), an enzyme utilizing L-aspartic acid (8); L-aspartic acid antagonizes the antifungal activity of DL-alanosine versus *Candida albicans* (9); and L-glutamic acid decarboxylase is inhibited by DL-alanosine in a competitive manner (10).

In our studies with murine lymphoblasts, it was the amides of the dicarboxylic acids which best antagonized the active transport of DL-alanosine. By contrast, in this system the dicarboxylic acids themselves were inactive.

Further studies on the mechanism by which DL-alanosine is metabolized, attempts to identify the principal metabolic products, and an evaluation of the antitumor activity of metabolites will be presented in future papers in this series.

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Phase II Trial of Baker's Antifol in Metastatic Sarcoma [1,2]

J. Tate Thigpen,[3,*] Robert M. O'Bryan,[4] Robert S. Benjamin,[5] and Charles A. Coltman, Jr[6,7]

SUMMARY

Iv Baker's antifol (BAF) (250 mg/m²/day × 3 consecutive days) was administered to 34 patients with metastatic sarcoma. All patients had received extensive prior therapy including prior chemotherapy and had progressive disease at the start of the study. Liver and renal functions were normal in all patients. Of 29 patients evaluable for response, 25 demonstrated progressive disease and four had stable disease for periods of from 1 to 6 months. No objective responses were observed. The other five patients died from 3 to 12 days after initiation of therapy. Toxicity included myelosuppression of significant degree in nine patients, gastrointestinal effects of nausea and vomiting in seven, stomatitis in three, and dermatitis in four. Most toxicity was mild to moderate, although one drug-related death due to marked myelosuppression was seen. In conclusion, BAF is considered to be insignificantly active in the secondary treatment of metastatic sarcomas at the dose and schedule studied.

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Baker's antifol (BAF), one of several dihydro-*S*-triazines synthesized by Dr. B. R. Baker, is a cycle-specific agent which inhibits DNA synthesis by reversible inhibition of dihydrofolate reductase (1). In preclinical studies, BAF proved highly effective against the ascites form of murine Walker 256 carcinosarcoma and against Dunning leukemia but was relatively ineffective against L1210 leukemia (2). Subsequent phase I trials at the M. D. Anderson Hospital suggested that a therapeutic effect might be achieved by courses given every 3 weeks at doses > 100 mg/m²/day × 5 days with acceptable toxicity (3,4). Additional data from the M. D. Anderson Hospital suggested that decreased toxicity with no sacrifice of therapeutic efficacy could be achieved with a dose of 250 mg/m²/day × 3 consecutive days (5). Responses in the phase I trial included one patient with synovial cell sarcoma, the only sarcoma patient in the study (4). The current study proposed to define the activity of the drug as a single agent in the treatment of metastatic soft tissue and bony sarcomas.

MATERIALS AND METHODS

At 13 member institutions of the Southwest Oncology Group, 34 adult patients with biopsy-proven metastatic sarcoma were evaluated with regard to history of prior treatment, performance status, extent of metastatic disease, hematologic parameters, and liver and renal functions. Tumor histology

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[3]Section of Oncology, Department of Medicine, University of Mississippi School of Medicine, Jackson.

[4]Division of Oncology, Henry Ford Hospital, Detroit, Mich.

[5]Section of Clinical Pharmacology, Department of Developmental Therapeutics, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston.

[6]Hematology-Oncology Service, Wilford Hall USAF Medical Center, Lackland AFB, San Antonio, Tex.

[7]This study was performed by the Southwest Oncology Group (SWOG). The following institutions participated in the study (the number of patients is in parentheses): Henry Ford Hospital (9); The University of Texas M. D. Anderson Hospital and Tumor Institute (7); Wilford Hall USAF (3); Kessler USAF (2); Scott and White Clinic (2); University of Oklahoma (2); University of Utah (2); Wayne State University (2); Northwestern University (1); Providence Hospital (1); University of Arkansas (1); University of Kansas (1); and University of Mississippi (1).

*Reprint requests to: Dr. J. Tate Thigpen, University of Mississippi Medical Center, 2500 N State St, Jackson, Miss 39216.

TABLE 1.—Histologic types of sarcomas entered in the study

Histologic type	No. of patients
Soft tissue sarcomas	28
Leiomyosarcoma	8
Fibrosarcoma	3
Synovial cell sarcoma	3
Liposarcoma	4
Mesothelioma	3
Angiosarcoma	1
Lymphangiosarcoma	1
Neurofibrosarcoma	1
Mixed mesodermal sarcoma	1
Undifferentiated sarcoma	3
Bony sarcomas	6
Osteosarcoma	2
Chondrosarcoma	1
Giant cell sarcoma	1
Ewing's sarcoma	2

TABLE 2.—Prognostic characteristics of patients studied

Prognostic factor	No. of patients
Prior treatment	34
Surgical resection	25
Radiotherapy to primary	15
Chemotherapy	34
Performance status (Karnofsky)	34
80%–100%	7
50%–70%	22
< 50%	5
Extent of disease	34
Local extension only	12
Pulmonary metastases	17
Hepatic metastases	3
Skeletal metastases	1
Pulmonary and brain metastases	1

(table 1) included a variety of soft tissue and bony types. All patients had received extensive prior therapy (table 2). No patient was started on BAF prior to recovery from effects of previous treatment. According to the initial performance status on the Karnofsky scale, most patients were symptomatic and partially bedridden. Most patients had extensive disease at the time of entry in the study. All patients entered in the study were required to have a granulocyte count of $> 2000/\mu l$, a platelet count of $> 100,000/\mu l$, and normal renal and hepatic functions as measured by BUN, creatinine, SGOT, and bilirubin.

All patients initially received BAF at a dose of 250 mg/m²/day as a 30-minute to 1-hour iv infusion in 50–100 ml of 5% dextrose in water or normal saline for 3 consecutive days. Drug courses were repeated at 3-week intervals. No dose escalation was permitted because of the risk of severe dermatitis at higher doses. Dose reduction by 33% was required if the lowest recorded granulocyte count was $< 1000/\mu l$, if the lowest recorded platelet count was $< 50,000/\mu l$, or if there was severe dermatitis, stomatitis, serious infection, or hemorrhage. Leukocyte, platelet, and differential counts were performed weekly. A patient was considered to be evaluable for response if he received one drug course and survived 3 weeks; he was considered to be evaluable for toxicity if he completed one 3-day course of drug regardless of subsequent survival.

Response was defined as follows: *complete*—disappearance of all evidence of disease; *partial*—a > 50% reduction in the product of perpendicular diameters of each measurable lesion; *progressive disease*—a > 50% increase in the product of perpendicular diameters of any measurable lesion or the appearance of any new lesion; and *stable disease*—< 50% regression or progression of lesions. Responses or disease stabilization were recorded only if the observed status persisted for > 1 month.

RESULTS

Of the 34 patients receiving one or more courses of BAF, 29 were considered to be evaluable for response. In the five patients not evaluable, death occurred from 3 to 12 days after administration of the first dose of BAF. Among the 29 patients evaluable for response, an average of 2.4 cycles of therapy were given per patient; 18 of the 29 received two or fewer cycles of therapy. No complete or partial responses were observed. Twenty-five of the 29 patients were removed from the study because of progressive disease during therapy. Four patients demonstrated stable disease for ≥ 1 month with the longest period of stabilization being 6 months in a patient with a peritoneal mesothelioma metastatic to the lung. Other stabilizations were seen in a retroperitoneal liposarcoma with extensive local spread, a chondrosarcoma of the ear metastatic to the lung, and a fibrosarcoma of undetermined primary metastatic to the lung.

All patients entered in the study were considered to be evaluable for toxicity (table 3). Nine patients developed significant myelosuppression. The lowest granulocyte counts were noted between Days 5 and 10 (median, Day 7) after the initiation of a drug course with rapid recovery thereafter. The lowest platelet counts were noted between Days 6 and 14 (median, Day 10) after initiation of drug administration with rapid recovery thereafter. One of the nine patients experienced fatal neutropenia and throm-

TABLE 3.—Adverse effects experienced in the study population

Adverse effects	No. of patients
Myelosuppression	9
Neutropenia (cells/μl)	7
Nadir, 500–1000	4
Nadir, 250–500	2
Nadir, < 250	1
Median nadir, Day 7 (range, Days 5–10)	
Thrombocytopenia (cells/μl)	4
Nadir, 50,000–75,000	2
Nadir, 25,000–50,000	1
Nadir, < 25,000	1
Median nadir, Day 10 (range, Days 6–14)	
Gastrointestinal	10
Nausea and vomiting	7
Severe	2
Stomatitis	3
Severe	1
Dermatologic	4
Severe	1

bocytopenia on Day 6 after initiation of therapy and was the only drug-related death in the study population. Ten patients experienced gastrointestinal toxicity, but only three of these had severe problems. Only four patients demonstrated dermatologic effects, and only one of these was a severe dermatitis; this confirms the findings of the M. D. Anderson group when a 3-day schedule was used.

DISCUSSION

BAF was selected for clinical trial as the most promising of a group of "non-classic" folate antagonists which differ, both in structure and in mode of handling by cells (6), from currently available folate antagonists such as methotrexate (1). The current study was undertaken to determine whether BAF would be a new active drug in the treatment of metastatic sarcomas. This possibility is suggested by extrapolation from data on the use of high-dose methotrexate plus citrovorum factor rescue (7) as well as from phase I data on BAF itself (3,4).

Results of this trial in sarcoma patients were disappointing because no evidence of drug activity was demonstrated. Although it is true that all patients had received extensive prior therapy and that almost 70% of the patients were symptomatic and partially bedridden, some evidence of drug activity should have been seen if indeed the drug has activity in sarcomas. Myelotoxicity of a significant degree was observed in nine of 34 patients treated (26%). This plus the unpredictable nature of toxicity seen with BAF make dose escalation potentially dangerous. On the basis of these data, BAF appears to be an insignificantly active single drug in the secondary treatment of metastatic sarcomas at the dose and schedule tested.

Toxicity of BAF in this study was similar to that noted in phase I trials (3,4) and was manifested as effects on bone marrow, skin, and gastrointestinal mucosa. One drug-related death occurred in a patient who became severely leukopenic and thrombocytopenic on Day 6 following initiation of therapy and died of apparent sepsis. Hepatic and renal functions were normal in this patient as determined by SGOT, alkaline phosphatase, bilirubin, BUN, and creatinine. The patient had received prior radiotherapy to the right anterior chest wall and axilla and prior chemotherapy consisting of vincristine, actinomycin D, and cyclophosphamide for 3 months and adriamycin plus DTIC for 3 months. These facts were similar for other patients studied. An autopsy failed to reveal a specific cause of death, although the patient did demonstrate pulmonary and skeletal metastases, a hemorrhagic pleural effusion, pneumonitis, and stress ulceration of the stomach with bleeding. Thus, no specific aspect of this case could be identified to account for the observed fatal toxicity. Five other patients developed severe myelotoxicity and stomatitis. In none of these five patients could factors be identified to account for the observed severity of toxicity. In all five, liver function as determined by the tests outlined above was normal. There was no evidence of cumulative toxicity of any sort in this study, but the duration of therapy in most patients was short.

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Selective Tumor DNA Synthesis Inhibition: In Vivo Prodrug Activation by an Exogenous Enzyme [1]

B. Tschiersch,* K. Schwabe, G. Sydow, and A. Graffi [2]

SUMMARY

Using the combination of α-L-arabinofuranosidase from *Aspergillus niger* with β-peltatin A-α-L-arabinofuranoside, the selective effect of a new cancer chemotherapy method based on a pH-dependent activation of cancerostatic prodrugs by *exogenous* enzymes was studied. In comparative experiments the selectivity of prodrug activation was measured by ^{3}H-thymidine incorporation in tumor and normal tissues of CBA mice inoculated im with the transplantable mammary carcinoma, MA-21224. The results show that this special type of carrier principle may lead to a higher degree of selectivity than the usual direct application of cancerostatic drugs.

[Cancer Treat Rep 61:1489–1493, 1977]

In recent years, several investigators have tried to utilize the normal body enzymes, also existing in higher or lower concentration in tumor tissues, or the pH difference between tumor and normal tissue, enhancable by application of glucose, to increase the selectivity of the cancerostatic effect (1–3). One of the well-known examples is the acid phosphatase of the prostatic carcinoma (4,5). With a few exceptions, these experiments have not yielded the expected success because of several limiting factors, eg, the enzyme concentration in the tumor is too low or the pH dependence of enzyme activity is too small. We have tried to overcome these difficulties by our method—the use of exogenous enzymes. Our premise is that the pH of the intercellular space of a tumor is slightly lower than the pH of the intercellular space of most normal tissues (especially after additional glucose treatment). We have prepared a drug derivative which is "activated" by a highly pH-dependent exogenous enzyme. Simultaneous administration of enzyme plus drug derivative may lead to preferential formation of active drug at tumor sites. We have tested this hypothesis by comparison of apparent inhibition of DNA synthesis by drug alone versus drug derivative plus activating enzyme and by tumor chemotherapeutic experiments.[3]

In several preceding papers and in connection with results of preliminary experiments, we have reported the possibility of activating prodrug in a selective pH-dependent reaction by exogenous enzymes in tumor tissues (6–11).[4] In these experiments we have used drug derivatives synthesized from well-known cancerostatics or other compounds with cytostatic activity by glycosidation (6–18). The enzymes used do not occur in the human body.

The preconditions of this new concept, ie, the enzyme qualities, the type of cancerostatic carrier, and other requirements, were published earlier in detail (6–11,13–21).[3] In this paper we would like to report on the results obtained with a combination of α-L-arabinofuranosidase and β-peltatin A-α-L-arabinofuranoside (fig 1) using biochemical methods.

METHODS AND MATERIALS

Drugs

The prodrug, β-peltatin A-α-L-arabinofuranoside, was synthesized from β-peltatin A, a lignan deriva-

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[2]Central Cancer Research Institute, Academy of Sciences of GDR, Berlin, German Democratic Republic.

*Reprint requests to: Dr. B. Tschiersch, Central Cancer Research Institute, Academy of Sciences of GDR, DDR-1115 Berlin-Buch, Lindenberger Weg 70, German Democratic Republic.

[3]Graffi A, Arnold W, Sydow G, et al. Experiments to increase the selectivity of tumor chemotherapy by means of in vivo-activation of transport forms of cancerostatics by exogenous enzymes. Submitted to *Cancer Treatment Reports*, 1977.

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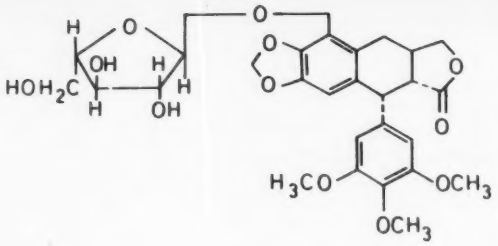

FIGURE 1.—Structure of β-peltatin A-α-L-arabinofuranoside.

tive with high antimitotic activity and a constituent of the natural product podophyllinum, by a modified Koenigs-Knorr reaction (17,18). This glycoside is largely detoxified and not splitable and thus is not activated by the body's own enzymes. However, with the special arabinosidase it is highly cleavable in the weakly acidic pH region.

The free β-peltatin A, the aglycone of our prodrug, and cyclophosphamide were chosen as reference substances in comparative experiments. In animal experiments the prodrug, the aglycone, and the cyclophosphamide were given sc in an isotonic NaCl solution containing 10% dimethylsulfoxide.

The comparative studies on DNA synthesis inhibition were carried out using ^{3}H-thymidine (specific activity, 28.3 curies/mM; UVVVR, Prague, Czechoslovakia) and the radioactive material (10 microcuries/mouse) was also given in an isotonic NaCl solution by iv injection.

The enzyme, α-L-arabinofuranosidase (specific activity, 15 IU/mg protein), was isolated from *Aspergillus niger* as described by Graffi (7) and Butschak et al (12,22). It was given in a dose of 15 IU/mouse in an isotonic NaCl solution by iv injection.

Animals

Female CBA mice weighing 18–20 g were used in these experiments. The mice were divided into groups of six to eight animals, and each experiment was repeated twice or more. The solid tumor (the formerly spontaneous mammary carcinoma MA-21224, found in 1962 and now existing in the 270th to 300th transplantation generation, a medullary and partly tubular growing adenocarcinoma),[5] was transplanted im by trocar implantation, and reached an average weight of 120 ± 28 mg within 9 days when the biochemical experiments were started.

[5]The histologic classification of the tumor by Dr. W. H. Mehnert is gratefully acknowledged.

Experimental Procedure

In the combination experiments the enzyme was administered first by iv injection and then, at different intervals of time, the prodrug was given by sc injection. The degree of activation proved by an optimal dosage of enzyme and of prodrug (optimal in regard to their ineffectiveness when given alone) is dependent mainly on the interval between application of these two components. In preliminary experiments, it was shown that this interval ranged from 2 to 8 hours. Using longer intervals, there is no sufficient enzyme activity in this model for in vivo prodrug activation measurable by the effect on ^{3}H-thymidine incorporation into DNA.

In all the combination experiments a solution of 100 mg of glucose per animal was given sc 1 hour before the injection of the prodrug or the reference drug to lower the pH value by increasing glycolysis in the solid tumors, as has been shown by several authors (20,23–25). Because of our success using this pH-lowering technique to enhance selectivity in other models (7)[6] and in the β-xylosidase plus 6-mercaptopurine-*S*-β-D-xylopyranoside combination system (21), we have optimized all our subsequent combination experiments with applications of glucose.

Other preceding experiments (21) show that the time of maximal inhibition of DNA synthesis is dependent on the type of drug. In our animal systems, antimetabolites, eg, 6-mercaptopurine, reached this maximum after 2 hours (21); antimitotics, eg, β-peltatin A, after 16 hours (table 1); and alkylating agents, eg, cyclophosphamide, after 24 hours in the different types of tissues. Thus, in our experiments, the ^{3}H-thymidine incorporation was always measured at the point of maximal DNA synthesis inhibition. Therefore, the ^{3}H-thymidine (10 microcuries) was given by iv injection 16 or 24 hours after application of the antimitotic or alkylating agent. One hour later the mice were killed and the tissues were homogenized and extracted for DNA isolation as described by Wunderlich et al (26). The radioactivity was measured in a Tricarb Scintillation Counter. The results are given in cpm/mg DNA.

RESULTS AND DISCUSSION

The experiments had to provide an answer to the two basic questions: Can this special prodrug be activated in vivo by the exogenous enzyme, as shown in other systems (7,21),[6] and is the action of the cancerostatic agent by this method more selec-

[6]*See* footnotes 3 and 4.

TABLE 1.—Time course of DNA synthesis in MA-21224 and normal tissue of CBA mice after receiving β-peltatin A (0.2 mg) or a combination of α-L-arabinofuranosidase (15 IU) and β-peltatin A-α-L-arabinofuranoside (0.5 mg) at 4-hr intervals*

	Hrs after β-peltatin A dosage				
	1	3	6	16	24
Tumor	24	13	9	1	25
Intestine	100	78	42	30	93
Spleen	57	16	9	4	27
Kidneys	73	44	15	7	19
Liver	63	45	34	11	58
	Hrs after combination dosage				
	2	6	16	24	
Tumor	15	7	1	36	
Intestine	41	36	28	81	
Spleen	68	45	24	48	
Kidneys	57	42	29	69	
Liver	54	52	31	59	

*Results are given in % of the ^{3}H-thymidine incorporation of untreated control animals.

tive than that afforded by conventional direct application of the substance? In this connection extensive studies and comparison treatments with combinations of α-L-arabinofuranosidase plus β-peltatin A-α-L-arabinofuranoside, and β-peltatin A or cyclophosphamide in different dosages, have been done.

A single iv injection of 15 IU of enzyme, the usual dose in most of our experiments, was well tolerated and had little or no effect on DNA synthesis (table 2). The effect of the prodrug alone on DNA synthesis is dependent upon the dose used. The dose selected in these experiments (0.5 mg given as a single sc injection) was chosen so that the effect of the prodrug alone was absent or negligible (table 2).

A combination of these two relatively nontoxic components, 15 IU of enzyme and 0.5 mg of prodrug, given at 4-hour intervals, produces a strong inhibition of DNA synthesis in the tumor. In all the normal tissues lower effects are seen (table 2). The degree of prodrug activation is variable and depends on the time interval between enzyme and prodrug application. The results of the same dosage of enzyme (15 IU) and of prodrug (0.5 mg), but given at 6-hour intervals, are shown in figure 2. Therefore, a great number of variations in the application form are possible with this method.

In our comparison of treatments, only variations of prodrug dosage and of the time interval were examined, together with different dosages of the reference substances, and we tried to achieve an identical inhibition of tumor DNA synthesis by different treatments. An example is given in figure 3. When comparing the action of a combination of 15 IU of enzyme and 0.5 mg of prodrug given at 4-hour intervals with the action of 0.2 mg of β-peltatin A given alone we found that the two methods produce a completely identical inhibition of tumor DNA synthesis but, in the inhibition of DNA syn-

TABLE 2.—DNA synthesis in MA-21224 and normal tissues of CBA mice after injection of α-L-arabinofuranosidase (15 IU), β-peltatin A-α-L-arabinofuranoside (0.5 mg), or a combination of both components given at 4-hr intervals*

	Control	Enzyme	Prodrug	Combination
	50	40	20	18
		(*No. of animals*)		
Tumor	11.2 ± 0.6	9.3 ± 0.5	9.3 ± 1.0	0.13 ± 0.03
Intestine	12.1 ± 0.6	10.7 ± 0.8	11.8 ± 0.7	3.39 ± 0.45
Spleen	7.1 ± 0.5	8.2 ± 0.7	5.0 ± 0.5	1.74 ± 0.20
Kidneys	1.2 ± 0.1	1.0 ± 0.2	0.7 ± 0.1	0.34 ± 0.14
Liver	1.4 ± 0.2	1.1 ± 0.1	1.0 ± 0.2	0.43 ± 0.13

*Results are given in cpm/mg DNA × 10^3.

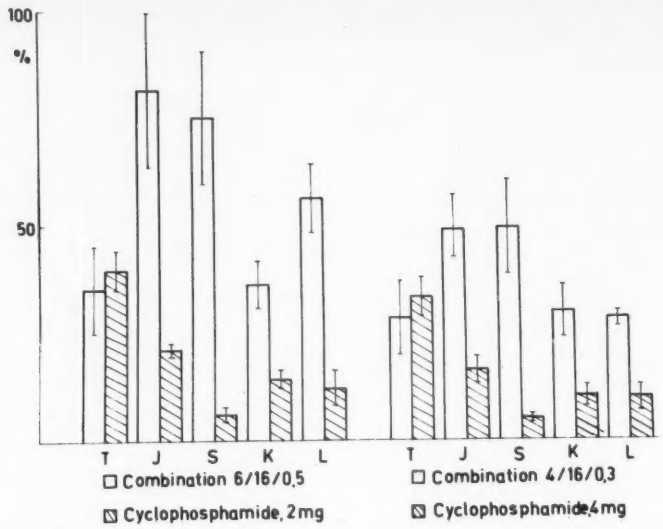

FIGURE 2.—DNA synthesis in MA-21224 (T), intestine (I), spleen (S), kidneys (K), and liver (L) of CBA mice after application of (*a*) a combination of α-L-arabinofuranosidase (15 IU) and β-peltatin A-α-L-arabinofuranoside (0.3 or 0.5 mg) at 4- or 6-hr intervals before the prodrug, or (*b*) cyclophosphamide (2.0 or 4.0 mg). ^{3}H-thymidine was given 16 hrs after the combination. Results are given in % of the DNA synthesis in untreated control animals.

thesis in the normal tissues, there is a distinct advantage favoring the combination method, especially for spleen and kidneys (fig 3).

We have also included in our experiments the most important clinically used cytostatics, for example, cyclophosphamide, and similar advantages for our therapeutic method are observable when comparing combinations of enzyme and prodrug with this compound in different dosages (fig 2).

In general, the selectivity gain resulting from the combination of an enzyme and a transport form is always present in the different models, but its level varies from one model to the other (6–10,21).[7] Our own biochemical experiments with α-L-arabinofuranosidase and β-peltatin A-α-L-arabinofuranoside, designed to verify this concept, have provided evidence that a selectivity gain can be achieved in this way also. The results show a high correspondence with those obtained in therapy experiments with the same combination of enzyme plus prodrug.[7] Therefore, it is very probable that in our experiments the interference with incorporation of thymidine into DNA actually represents interference with DNA synthesis.

Some factors, for instance, the insufficient detoxification of the prodrugs or the still present residual activity of some enzymes in the physiologic pH region 7.3–7.4, have pronounced negative effects. The ideal combination consists of a practically inactive transport form and an enzyme being completely inactive at pH 7.4.

In the search for methods to improve cancer chemotherapy, our concept has opened new possibilities. The use of a variety of exogenous enzymes in combination with the tailor-made cancerostatic prodrugs could possibly provide a broad application for this specialized form of the carrier principle.

[7] *See* footnote 3.

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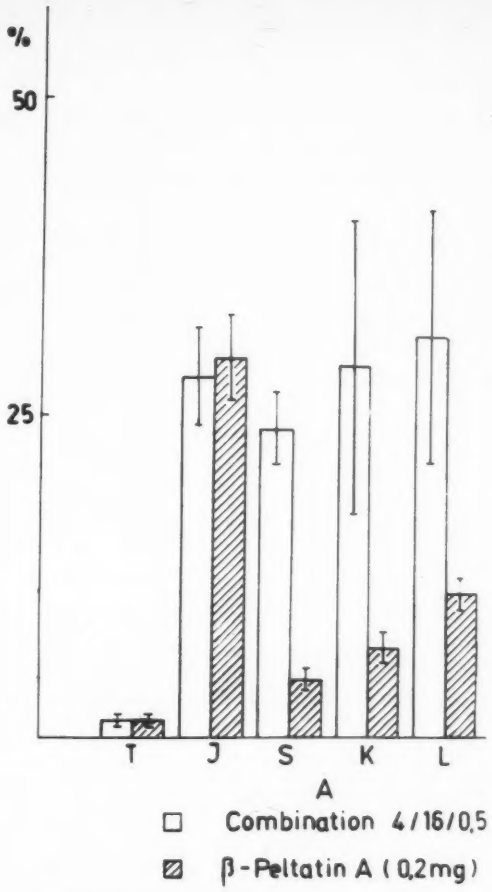

FIGURE 3.—DNA synthesis in MA-21224 (T), intestine (I), spleen (S), kidneys (K), and liver (L) of CBA mice after application of (*a*) a combination of α-L-arabinofuranosidase (15 IU) and β-peltatin A-α-L-arabinofuranoside (0.5 mg) at 4-hr intervals, or (*b*) β-peltatin A (0.2 mg). ^{3}H-thymidine was given 16 hrs after the combination or the β-peltatin A dose. Results are given in % of the DNA synthesis in untreated control animals.

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Intermittent High Single-Dose Ftorafur: Phase I Clinical Trial With a Pharmacologic-Toxicity Correlation [1,2,3]

Stephen W. Hall,[4] Manuel Valdivieso,[4] and Robert S. Benjamin [4,5,6,*]

The fluorinated pyrimidine compounds are antitumor agents acting as antimetabolites for natural nucleosides. 5-Fluorouracil (5-FU) and 5-fluoro-2'-deoxyuridine are members of this group, acting predominantly against adenocarcinomas of the breast and gastrointestinal (GI) tract.

The synthesis of ftorafur, the 1-tetrahydrofuranyl derivative of 5-FU, was reported by Hiller et al from the Soviet Union in 1968 (1). Since then, experimental studies with this drug have been performed comparing it with 5-FU (2). Ftorafur appears to be less toxic to hematopoietic cells and five to six times less toxic to mice than 5-FU as determined by lethal dose studies (2). Most in vitro studies have failed to show biologic activity of unchanged ftorafur and suggest that the antineoplastic activity of ftorafur is due to release of 5-FU in vivo (3). Studies in man showed ftorafur to be less toxic and as effective as 5-FU against colon and breast carcinoma (4). Early phase I clinical trials utilizing a daily infusion schedule of 1–2.5 g/m^2 × 5 days revealed a 17% antitumor response (complete and partial remission) in evaluable patients and significant GI and constitutional toxicity (5). Similar toxicities were found by others using a 5–10-day schedule (6). Our preliminary clinical pharmacology studies demonstrated a long plasma half-life for ftorafur and its metabolites and prolonged therapeutic levels of 5-FU, suggesting that an intermittent single-dose schedule of drug administration might be appropriate (7). This report is the result of such a phase I clinical trial in patients with disseminated neoplasms. In addition, pertinent pharmacology studies that correlate with clinical toxicity are reported.

PATIENTS AND METHODS

Fourteen adult patients with disseminated neoplasms were entered in the study. There were eight men and six women ranging in age from 32 to 79 years (median, 60 years). The diagnoses of patients studied included three gastric, two lung, two breast, two pancreatic, one prostate, one jejunal, and one colon carcinoma, one carcinoid tumor, and one melanoma. Five patients had not received prior chemotherapy and had GI tumors considered sensitive to fluoropyrimidines. Nine patients had received prior chemotherapy, two having received 5-FU, although they were not definitely refractory to it. Other prior drug therapy included adriamycin, cyclophosphamide, DTIC, methyl-CCNU, and pyrazofurin. Ftorafur was dissolved in 250–500 ml of 5% dextrose and water and given iv over 15 minutes to 2 hours. The patients received initial ftorafur doses of 3, 4, or 5 g/m^2 which were modified or repeated at 2–3-week intervals depending on toxicity from the previous course (table 1). Patients were hospitalized and closely evaluated with tests for vital signs and neurologic examinations for the first 24 hours after the initial dose. Hematologic parameters were checked at least twice weekly. One patient received only a 50-mg/m^2 tracer dose of ftorafur for pharmacology studies. Pharmacology studies were performed using ^{14}C-labeled ftorafur in selected patients with cerebrospinal fluid (CSF) pharmacology obtained in three. Drug determinations were done as previously described utilizing radiochemical and

TABLE 1.—No. of courses given at each dose level of ftorafur

Dose (g/m^2)	No. of courses given—	
	Initially	Subsequently
3	1	3
4	2	6
5	10	9

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[3]Ftorafur: NSC-148958; CAS reg. No. 17902-23-7; uracil, 5-fluoro-1-(tetrahydro-2-furyl)-.

[4]Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[5]Junior Faculty Fellow of the American Cancer Society.

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*Reprint requests to: Robert S. Benjamin, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

TABLE 2.—Myelosuppressive toxicity of ftorafur*

Dose (g/m^2)		Wbc count (cells × $10^3/\mu l$)	Absolute granulocyte count (cells × $10^3/\mu l$)	Platelet count (cells × $10^3/\mu l$)
3	No. of courses	2	2	2
	Before ftorafur	8.6 and 8	4.8 and 4.8	680 and 580
	After ftorafur (lowest)	6.3 and 6.3	4.2 and 2.5	596 and 448
4	No. of courses	6	6	6
	Before ftorafur	6.1 (4–7.2)	3.4 (2.4–4.2)	360 (250–698)
	After ftorafur (lowest)	5.4 (5.2–6.6)	3 (2.1–3.9)	332 (260–535)
5	No. of courses	14	14	14
	Before ftorafur	7 (3.6–13.2)	4.7 (2.5–11)	345 (61†–586)
	After ftorafur	7.5 (3.2–11.9)	4.2 (0.8†–8.4)	458 (59†–559)

*Toxicity before and after a dose of ftorafur is expressed with median and range (in parentheses).
†Occurred in a patient with breast carcinoma and bone marrow involvement by tumor.

chromatographic techniques, which allowed separation and quantitation of ftorafur, 5-FU, and some other metabolites (8).

RESULTS

Response

Although an objective of this study was to determine the maximum tolerated dose and pattern of toxicity for ftorafur administered by intermittent single doses, it was possible to evaluate ten patients for antitumor effect. There were no patients with evidence of significant tumor regression; however, five patients with previously progressive disease had stabilization of tumor growth lasting from 6 to 15 weeks. This disease stabilization was seen in patients with GI or breast carcinomas, tumors most likely to be sensitive to 5-FU or its derivatives.

Toxicity

Hematologic toxicity was evaluable in a total of 22 courses at doses of 3–5 g/m^2 (table 2). If we exclude one patient at a dose of 5 g/m^2 who had breast carcinoma with extensive bone marrow involvement, there was no clinically significant decrease in the wbc or platelet counts. Nonhematologic toxicity was much more prominent than myelosuppressive toxicity and was dose limiting. GI and central nervous system (CNS) reactions were the most frequent and dose related (table 3). Lethargy, confusion, ataxia, and dizziness were unacceptable at 5 g/m^2 as manifestations of CNS toxicity. All toxicities were acute, having their onset during or soon after drug administration and resolving or markedly improving within 4–24 hours. Although nausea and vomiting were seen in 100% of the patients and were moderately severe in 75% at the 5-g/m^2 dose, they were acceptable in a majority of the patients because of their short duration. Chest tightness and flushing were occasionally seen, appeared to be related to rapid drug infusion, and quickly disappeared or significantly improved if the drug infusion was stopped or the rate of infusion was slowed. At the 4-g/m^2 dose two patients experienced CNS reactions. One reaction included mild anxiety and headache requiring no therapy and the other reaction included apparent hallucinations. Both reactions appeared related to very rapid drug administration of < 30 minutes. At the 3-g/m^2 dose only two patients were treated, with nausea and vomiting the only toxicity seen.

Pharmacology Data

Preliminary pharmacology studies revealed rapid and high levels of ftorafur and 5-FU in CSF after drug administration which may explain the CNS toxicity (table 4). No other ftorafur or 5-FU metabolites were identified in the CSF. In a patient given 5 g/m^2 of ftorafur iv, CSF levels of ftorafur at 15–30 minutes were 20%–50% of the plasma levels showing very rapid entry of ftorafur into the CSF. The CSF level of ftorafur surpassed the plasma level by 4–5 hours after drug administration. CSF levels of 5-FU produced from ftorafur were similar and approached the simultaneous plasma level at 4–8 hours. Patient 2 (table 4) had a CSF sample obtained 6 hours after drug administration with a CSF level of ftorafur twice the plasma level. Patient 3 had received only a tracer dose (50 mg) of ftorafur and had a CSF level of total radioactivity twice the plasma level at 5 hours.

TABLE 3.—Other toxicities with single high doses of ftorafur*

	Dose (g/m²)		
	3	4	5
No. of evaluable patients	2	5	10
No. of evaluable courses	4	8	16
% patients with toxicity	50	50	100
% courses with toxicity	100	100	100
% nausea and vomiting	100	88	100
Severe	—	13	75
Moderate	100	75	25
% weakness	—	—	31 (50)
% chest tightness	—	—	13 (20)
% flushing	—	—	13 (20)
% diarrhea	—	—	6 (10)
% anorexia	—	—	6 (10)
CNS			
% lethargy and confusion	—	25 (40)	38 (60)
% ataxia and dizziness	—	—	19 (30)
% anxiety	—	13 (20)	13 (20)
% blurred vision	—	—	13 (20)
% headache	—	13 (20)	13 (20)
% hallucinations	—	13 (20)	—

*Toxicities are expressed in relation to the No. of treatments administered and to the No. of patients evaluable at each dose level (in parentheses).

TABLE 4.—CNS penetration of ftorafur

Patient No.	Dose	Time (hrs)	CSF-ftorafur level (μg/ml)	Plasma-ftorafur level (μg/ml)	CSF-5-FU level (μg/ml)	Plasma-5-FU level (μg/ml)
1	5 g/m²	1	55	152	1.0	2.7
		4	105	107	2.5	2.8
		8	104	71	2.3	2.4
		24	26	14	0.6	2.0
2	5 g/m²	6	193	100	1.7	1.4
3	50 mg/m²*	5	0.59	0.32	—	—

*Tracer dose only; values are total radioactivity expressed as ftorafur equivalents.

DISCUSSION

Ftorafur, as a single intermittent dose, produces extensive and unacceptable CNS and GI toxicity at 5 g/m², but is quite tolerable with minimal GI or CNS toxicity at 4 g/m². It is recommended that ftorafur be given at the 4-g/m² dose and repeated at approximately 14-day intervals. Rapid administration may cause acute reactions, particularly in the CNS or GI tract, which may be avoided or decreased by infusing the drug over ≥ 2 hours. There was no significant myelotoxicity in patients with normal bone marrow reserve; however, in patients with severely compromised marrow reserve due to tumor involvement or prior therapy, the drug effect should be monitored carefully with frequent hematologic examination. Many of our patients had received one or more chemotherapy regimens prior to ftorafur and did not have myelosuppression.

These pharmacology data show rapidly accumulating high levels of ftorafur and 5-FU in the CSF after ftorafur administration and most likely explain the CNS toxicity of the drug. Other investigators have found evidence of fluorocitrate and fluoroacetate in the brains of 5-FU-treated cats which may explain the neurotoxicity of that drug (9). In our patients we did not find other metabolites of

ftorafur or 5-FU in the CSF; however, other investigators have found 5-FU metabolites in the CSF equivalent to those in plasma after iv bolus 5-FU injection (10). Because the CNS toxicity of ftorafur may differ slightly from that produced by 5-FU (more CNS depression and/or stimulation as opposed to cerebellar abnormalities), the specific compound responsible may be different. The compound may be unchanged ftorafur, an intermediate metabolite between ftorafur and 5-FU, or the tetrahydrofuran moiety. It is also possible that ftorafur may influence the subsequent metabolism, activation, or degradation of 5-FU, permitting high concentrations of a specific metabolite in the CNS. Alternatively, the CNS effects may be related to the prolonged retention of ftorafur, 5-FU, or metabolites in the CNS, a finding not observed after bolus administration of 5-FU. The acute nausea and vomiting may also be related to the high CNS levels of ftorafur or its metabolites. Prolonged CNS levels of ftorafur may be particularly effective, however, in the treatment of intracranial tumors.

Plasma-5-FU levels produced by ftorafur after a single high dose are at least equivalent to those produced by a therapeutic 5-day continuous infusion of 5-FU (maximum tolerated dose, 1–1.1 g/m²/day), and in fact both regimens are nonmyelosuppressive and limited by GI toxicity (7). Using the 5-g/m² dose resulted in excessive CNS toxicity which could be substantially reduced by decreasing the dose to 4 g/m². The high single-dose regimen of ftorafur appears to produce less prolonged debilitation and more tolerable constitutional toxicity than successive ftorafur administration, since debilitation is primarily limited to the day of treatment. We have also seen more constitutional debilitation in patients receiving successive daily ftorafur in combination regimens than in those receiving the drug alone on the same schedule. It is possible that if the high single-dose ftorafur regimen was used in those combination regimens this toxicity might be decreased. We feel that the clinical pharmacology and toxicology support the continued investigation of ftorafur given in intermittent high single doses. To establish the antitumor effect of ftorafur by this schedule will require study of a larger number of patients in phase II–III studies. We are currently continuing our investigations of high-dose ftorafur in this context in other chemotherapy regimens.

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Passive Immunotherapy for Mouse Leukemias With Antisera of "Directed" Specificity: Synergism With the Action of Cyclophosphamide [1,2]

Arnold E. Reif,[3,*] Roger W. Li,[4] and Cynthia M. Robinson [5,6]

SUMMARY

Antileukemia sera with "directed" specificity are produced by immunization of rabbits with mouse leukemia cells admixed with normal antigen blocking (NAB) serum. Addition of NAB serum to the leukemia cells inhibits production of antibodies to normal cell components and directs specificity toward leukemia cell antigens. The resulting antileukemia serum (ALK-NABS) was not sufficiently potent to produce more than moderate therapy in the standard L1210 leukemia therapy assay. When given together with noncurative doses of cyclophosphamide (CTX), ALK-NABS acts synergistically. It is most effective when given early after injection of the leukemia cells and prior to injection of CTX. Daily repeated injections of a given dose are more effective than a single injection of that dose. Most important, small doses of ALK-NABS produce significant prolongation of lifespan in conjunction with CTX. Results of therapy for BW-A leukemia with ALK-NABS in conjunction with CTX were negative.

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The purpose of this study was to extend past attempts to use passive transfer of heterologous antisera for immunotherapy in a mouse model system. While specificity against leukemia cells is more easily attained with alloantisera, any eventual extension of this work from mouse to man will be far more convenient with heterologous antisera. For this reason, heterologous antisera are used here.

Previous studies of conventionally prepared heterologous antileukemia sera (ALKS) in mouse model systems have shown only modest in vitro specificity for leukemia cells relative to normal lymphocytes (1–3). In vivo therapy is ineffective unless the leukemia grows slowly or a small inoculum of leukemia cells is given (4–6). Improved results have been obtained with ALKS prepared by immunization of rabbits with leukemia cells admixed with normal antigen blocking (NAB) serum prepared by immunizing rabbits with normal mouse lymphoid cells. The resulting antileukemia normal antigen blocking serum (ALK-NABS) is less toxic than the conventional ALKS, but is still only mildly effective (7–9). Therefore, the need for a search for synergism with established chemotherapeutic agents has become apparent.

In our last study with L1210 leukemia, we obtained significantly higher survival of mice injected on Days 1–4 with ALK-NABS (10). In contrast to our previous results with conventional ALKS (6), best results were obtained at doses well below the maximum tolerated dose of ALK-NABS. The addition of guinea pig complement was not helpful. Results with an isogenic AKR leukemia (BW-A) were far less encouraging.

The present experiments report synergism between immunotherapy with ALK-NABS and chemotherapy with cyclophosphamide (CTX). A brief report of these results has been made (11).

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[3]Mallory Institute of Pathology, Boston City Hospital, and Department of Pathology, Boston University School of Medicine, Mass.

[4]Yale University, New Haven, Conn.

[5]Rheumatology Laboratory, Tufts-New England Medical Center, Boston, Mass.

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*Reprint requests to: Dr. Arnold E. Reif, Boston City Hospital, Boston, Mass 02118.

MATERIALS AND METHODS

Animals, tumors, and cell suspensions.—DBA/2 and AKR mice were obtained from the Jackson Laboratory. L1210 leukemia and BW-5147 leukemia (converted by us into the ascites form and re-named BW-A) were transplanted in the respective strains of origin, namely DBA/2 and AKR. New Zealand white rabbits weighing between 6 and 9 lb were used for immunization. Single cell suspensions of splenic and leukemic lymphocytes were prepared as previously described (12), maintained at 4°C, and used within 2 hours.

In vitro assays of cytolytic potencies.—In vitro assays were performed in 0.3 ml volume (12). Each assay tube contained 10^5 viable lymphocytes, absorbed guinea pig complement at 10% final concentration, and serial doubling dilutions of antiserum. After incubation for 1 hour at 37°C, cells were classified as stained or unstained after addition of vital dye. Percent of stained cells was plotted against final concentration of antiserum (%) on semilogarithmic paper, and the cytolytic titer was read at 50% stained cells, with a correction for stained cells in the control tube. Results are expressed as "cytolytic potency," defined as 100/cytolytic titer; thus, an antiserum that has a cytolytic titer at 1.0% final concentration of antiserum has a cytolytic potency of 100. The cytolytic potency is also numerically equal to the final antiserum dilution at the titration point: an antiserum with a cytolytic titer of 1.0% or 1:100 final dilution has a cytolytic potency of 100. Cytolytic potency units have been defined as "cytolytic potency × volume (ml) of solution with that potency" (6).

Preparation of NABS.—The ALK-NABS used in the present experiments was prepared by the injection of leukemia cells admixed with NABS (*see* section below). Therefore, the NABS reagent had to be prepared first as follows. Two groups of three rabbits each were immunized by ten iv injections of 100 million spleen cells per rabbit per injection. Spleens taken respectively from DBA/2 and C3HeB/Fe mice were used. The latter strain was substituted for AKR to avoid the possibility that antisera might contain antibodies to the Gross virus which might cross-react with surface antigens on the BW-A cells; both AKR and C3HeB/Fe mice are H-2^k. Rabbits were bled on Days 8 and 9 after the last injection, and the sera were pooled by group and stored at –10°C. The cytolytic potencies of the above NABS reagents were as follows. The anti-DBA/2 NABS had cytolytic potencies of 2320 and 18,500 against DBA/2 splenic and thymic lymphocytes, respectively. The anti-C3HeB/Fe NABS had cytolytic potencies of 1430, 4550, and 4160 against AKR splenocytes, AKR thymocytes, and C3HeB/Fe thymocytes, respectively.

Preparation of ALK-NABS.—Separate antisera were prepared in groups of three rabbits for the two leukemias, L1210 and BW-A, under investigation. Each rabbit received eight iv injections spaced over 4 weeks. Each injection consisted of 50 million leukemia cells admixed for 30 minutes at room temperature immediately prior to injection with an amount of the appropriate NAB serum calculated to contain 3000 cytolytic potency units against splenic lymphocytes. For instance, 1.3 ml of the anti-DBA/2 NABS (potency 2320) was incubated with each 50 million L1210 cells prior to injection. As for NAB sera, pools for antisera to each leukemia were prepared and stored at –10°C. ALK-NAB sera were absorbed once with erythrocytes of the appropriate mouse strain, using washed erythrocytes obtained from 18 ml of whole blood to absorb 100 ml of antiserum. Prior to absorption, complement activity of the antisera was inactivated by the addition of 0.5 M disodium EDTA (pH 7.25, 3% of the volume of the antiserum) to remove divalent cations. For the absorption, antisera were mixed gently with cell suspensions for 1 hour at room temperature, then centrifuged at 3000 *g* for 15 minutes at 5°C. After absorption, divalent cations were restored by the addition of a mixture that contained 1.0 M $MgCl_2 \cdot 6H_2O$ and 0.3 M $CaCl_2$ (1% of the volume of the antiserum) (10). The cytolytic potencies of the absorbed antisera were determined against both normal lymphocytes and leukemia cells (table 1). Hemagglutination potencies of the absorbed sera were also determined by a previously described method (10). The specificity of the antisera was evaluated (*see* Results section) by means of the specificity ratio, which is defined as the ratio of potencies of an antiserum in its reaction against two antigens or complex antigens (10).

Therapy experiments.—Therapy experiments were begun by ip injection of freshly drawn sterile tumor cells, precisely standardized as to number, into isogenic recipients (Day 0). At stated times later, various treatment regimens were given, as specified in the protocol. Daily records of deaths were kept. In cases for which the cause of death was in doubt, autopsies were performed. Additional experimental details are described elsewhere (10).

RESULTS

In Vitro Specificity of Antisera

The cytolytic potencies of the ALK-NABS prepared against L1210 were 124 against this leukemia,

TABLE 1.—Potencies following absorption of sera used for therapy

Serum	Potencies determined by agglutination (rbcs) or cytolysis against—							
	DBA/2 rbcs	AKR rbcs	DBA/2 splenocytes	AKR splenocytes	DBA/2 thymocytes	AKR thymocytes	DBA/2 L1210 leukemia	AKR BW-A leukemia
NRS	4	<4	4.1	5.5	12.0	11.0	9.3	2.5
ALK-NABS vs L1210	96	80	194		590	850	124	
ALK-NABS vs BW-A	96	32	44*	50	2040	2240	122*	1030

*Single values. All other values are means of duplicate determinations.

194 against DBA/2 splenic lymphocytes, and 590 against DBA/2 thymocytes (table 1). The specificity ratios for the reaction of this antiserum against the L1210 leukemia relative to splenocytes and thymocytes were therefore 0.64 (124/194) and 0.21. The cytolytic potencies of the ALK-NABS prepared against BW-A leukemia were 1030 against this leukemia, 50 against AKR splenocytes, and 2240 against AKR thymocytes (table 1). The specificity ratios for the reaction of this antiserum against the BW-A leukemia relative to splenocytes and thymocytes were therefore 20.6 and 0.46. The low ratios of the specificity ratios for the above ALK-NAB sera indicate a relative lack of specificity against leukemia cells, as determined by immune cytolysis. Both the anti-L1210 and the anti-BW-A sera were produced using NABS prepared against the respective splenocytes; the low cytolytic titer of the anti-BW-A ALK-NABS when tested against splenocytes (and consequently the high BW-A/splenocyte specificity ratio) was probably obtained because this leukemia is an AKR thymic (T-cell) lymphoma, while L1210 is a B-cell leukemia. The hemagglutination potencies (table 1, columns 1 and 2) are relevant because single doses that contain > 150 potency units are acutely toxic in vivo (6).

Experiment 1

The purpose of this experiment (fig 1) was to look for synergism between the action of ALK-NABS and CTX. In this and subsequent experiments, the statistical significance of the data was determined by one-way analysis of variance; comparison between different groups was then performed with the t test. The Hewlett-Packard 9815A programmable calculator was used for these tests.

There was no statistically significant difference in survival times of the four control groups, which received either eight or five injections of saline, or guinea pig serum (GPS), or normal rabbit serum (NRS) plus GPS (A, B, C, and D, fig 1). When NRS was replaced by ALK-NABS (E and F), there was a significant increase in survival compared to the corresponding saline control groups B and A ($P < 0.01$), but there were no permanent survivors. Similarly, CTX given alone on Day 7 prolonged survival relative to control groups B or A ($P < 0.01$), but no mice survived (group G). Remarkably, the beneficial effect of CTX was reversed by addition of NRS plus GPS (group H: $P < 0.01$). However, when both ALK-NABS and CTX were given, all mice survived for > 90 days (group I). Unexpectedly, CTX was ineffective when given on Day 3 rather than on Day 7, and the addition of NRS plus GPS, or of ALK-NABS plus GPS, did not have any effect except that one survivor was obtained in the last group (fig 1, groups J, K, and L). Iv injection of bacillus Calmette-Guérin (BCG) was not effective in this experiment (groups M, N, and O, as compared to groups A, D, and E, respectively).

Experiments 2, 3, and 4

Because we had previously obtained data (10) that suggested that the anti-L1210 ALK-NABS reagent was effective at doses < 0.8 ml, as used in experiment 1, we investigated the use of much smaller amounts of this reagent in conjunction with CTX (figs 2–4).

First, we used one fourth the amount of ALK-NABS and GPS that was used in experiment 1 (fig 2). Given alone, ALK-NABS plus GPS in this quantity had no significant effect upon survival (B–H compared to A, fig 2). However, compared to group a, which was given CTX, statistically significant prolongation of lifespan was obtained for several groups that, in addition, received small amounts of ALK-NABS plus GPS: for groups b ($P < 0.05$), e ($P < 0.05$), f and g ($P < 0.01$), h ($P < 0.05$), and i ($P < 0.01$).

Even though no permanent survivors were obtained in this experiment, a significant effect was obtained in group g of experiment 2 (fig 2) with 0.067 ml of ALK-NABS per injection per 20-g mouse. It therefore seemed worthwhile to test the effectiveness of such a relatively minute dose of ALK-NABS in greater detail. This was done in experiments 3 and 4 (figs 3 and 4).

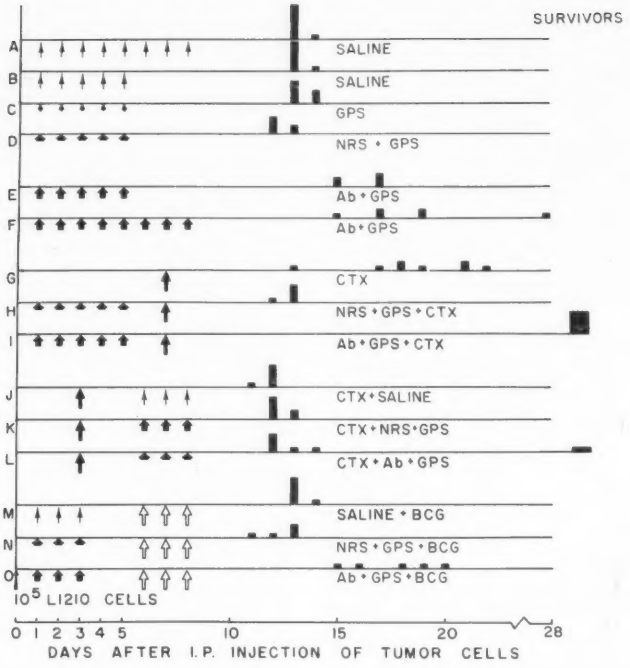

FIGURE 1.—Therapy experiment 1 with L1210 leukemia (transplant generation 37 following frozen storage) in DBA/2 mice. Leukemia cells ( ) were given ip on Day 0 for all groups (A–O) which are identified by a letter below their horizontal line. All therapy injections except BCG were given ip at stated times (arrows) from Day 1 to 8. For each injection per day per 19.0-g mouse: saline ( ) = 1.0 ml isotonic sodium chloride, GPS ( ) = 0.2 ml active GPS absorbed with DBA/2 rbcs (12), NRS + GPS ( ) = 0.8 ml NRS absorbed with rbcs plus 0.2 ml absorbed GPS, Ab + GPS ( ) = 0.8 ml ALK-NABS absorbed with rbcs plus 0.2 ml absorbed GPS, CTX ( ) = 100 mg/kg cyclophosphamide, BCG ( ) = approximately 5×10^6 viable organisms (0.6 mg) of University of Illinois BCG given iv on Days 6 and 7, and intradermally into 4 sites on Day 8. Each of the therapy injections (except BCG) was brought up to 1.0 ml with isotonic saline. Day of death is indicated by a solid bar of unit height per mouse. Survivors are indicated by a wider cross-hatched bar of the same unit height per mouse on the extreme right of the figure. Nos. of mice in different groups were A = 9, B = 8, C = 8, D = 6, E = 5, F = 6, G = 8, H = 5, I = 5, J = 6, K = 7, L = 7, M = 7, N = 5, O = 5.

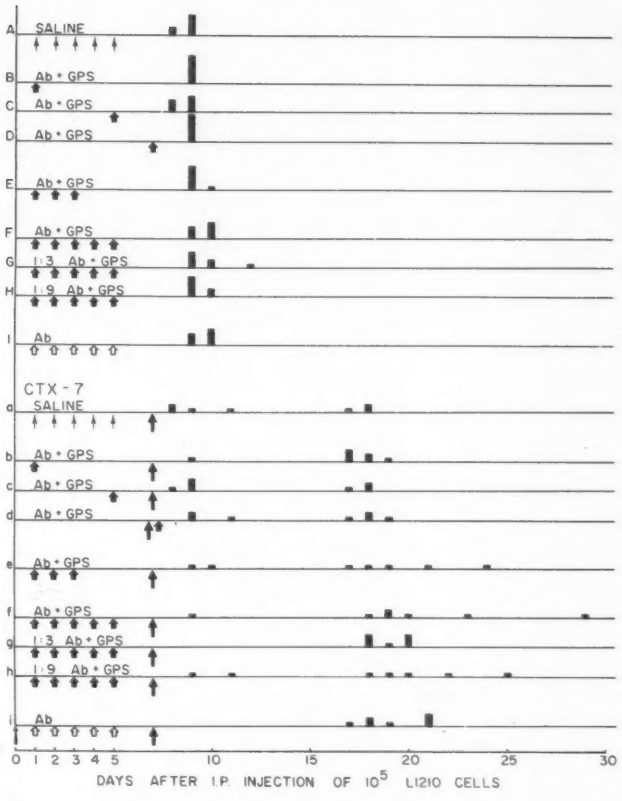
A SALINE
B Ab + GPS
C Ab + GPS
D Ab + GPS
E Ab + GPS
F Ab + GPS
G 1:3 Ab + GPS
H 1:9 Ab + GPS
I Ab
CTX - 7
a SALINE
b Ab + GPS
c Ab + GPS
d Ab + GPS
e Ab + GPS
f Ab + GPS
g 1:3 Ab + GPS
h 1:9 Ab + GPS
i Ab
0 1 2 3 4 5 10 15 20 25 30
DAYS AFTER I.P. INJECTION OF 10^5 L1210 CELLS

FIGURE 2.—Therapy experiment 2 with L1210 leukemia (transplant generation 2 following frozen storage) in DBA/2 mice. Leukemia cells were given ip on Day 0 for all groups (A–i) which are identified by a letter above their horizontal line. All therapy injections were given ip at stated times (arrows) from Day 1 to 7. For each injection per day per 20.0-g mouse: saline (↓) = 1.0 ml isotonic NaCl, Ab + GPS (⇩) = 0.20 ml absorbed ALK-NABS plus 0.05 ml absorbed active GPS (1:3 and 1:9 represent dilutions with isotonic saline), CTX (⇓) = 165 mg/kg cyclophosphamide, Ab (⇩) = 0.20 ml absorbed ALK-NABS. Each therapy injection was brought up to 1.0 ml with isotonic saline. Day of death is indicated by solid bar of unit height per mouse. There were 7 mice in each group.

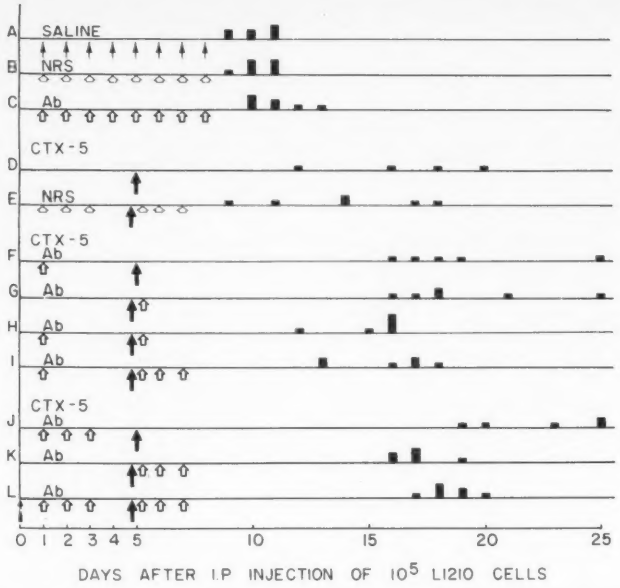

FIGURE 3.— Therapy experiment 3 with L1210 leukemia (transplant generation 5 following frozen storage) in DBA/2 mice. Leukemia cells were given ip on Day 0 for all groups (A–L) identified by a letter above their horizontal line. All therapy injections were given ip at stated times (arrows) from Day 1 to 8. For each injection per day per 20.0-g mouse: saline (↓) = 1.0 ml isotonic NaCl, NRS (○) = 0.067 ml absorbed NRS, Ab (⇧) = 0.067 ml absorbed ALK-NABS, CTX (↑) = 165 mg/kg cyclophosphamide. Day of death is indicated by solid bar of unit height per mouse. Nos. of mice in different groups were A = 7, B = 7, C = 7, D = 4, E = 6, F = 5, G = 6, H = 6, I = 6, J = 5, K = 6, L = 7.

In experiment 3, CTX was given on Day 5 instead of on Day 7 as in experiments 1 and 2. Antibody was given without the addition of GPS, as there was no evidence from experiments 1 and 2 that GPS was helpful in our assay system. Antibody, when administered without CTX, had no significant effect upon survival (groups C and A, fig 3). Of all the groups that received both CTX and antibody, only the group that received injections on Days 1, 2, and 3 (group J) showed prolonged survival ($P < 0.01$).

Experiment 4 was run simultaneously with experiment 3, the only difference being administration of CTX on Day 7, as in experiments 1 and 2. Interestingly, NRS was significantly deleterious ($P < 0.01$) when given in the time sequence tested (groups N and M, fig 4). The only experimental group given antibody plus CTX that showed significantly prolonged survival ($P < 0.05$) was group T, which received six injections of antibody (0.067 ml per injection).

Experiment 5

We had previously obtained negative results with anti-BW-A ALK-NABS therapy when this was administered without CTX chemotherapy (10). In experiment 5, we tested ALK-NABS in conjunction with CTX (fig 5). This experiment also proved to be negative: while CTX significantly prolonged survival, though also not dramatically (group B compared to A, $P < 0.05$), none of the experimental groups (C–H) given different amounts of ALK-NABS on Days 1–4 showed significantly prolonged survival over group B, which received CTX alone. The only positive result was one permanent survivor in the group given ALK-NABS as well as CTX plus BCNU (group J compared to group I).

Experiment 6

A comprehensive experiment to investigate the effect of low doses of anti-L1210 ALK-NABS on survival of DBA/2 mice inoculated with a wide range of doses of L1210 cells but not treated with CTX was then performed (fig 6). Compared to the saline-injected control groups, statistically significant prolongation of survival was obtained for the following groups: at 10^2 L1210 cells for groups D ($P < 0.05$), E and F ($P < 0.01$); at 3×10^2 cells for group M ($P < 0.01$); at 10^3 cells for no

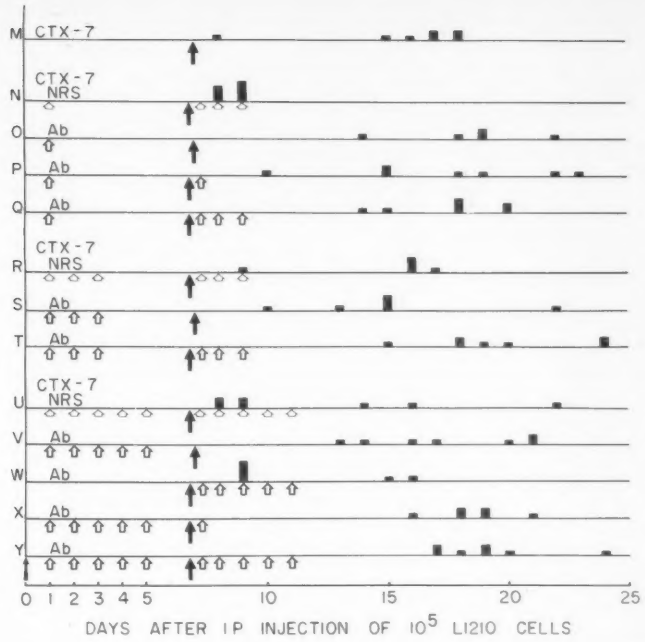

FIGURE 4.—Therapy experiment 4 with L1210 leukemia (transplant generation 5 following frozen storage) in DBA/2 mice. All injections as in fig 3. Nos. of mice in different groups were M = 7, N = 7, O = 5, P = 7, Q = 7, R = 5, S = 6, T = 7, U = 7, V = 7, W = 6, X = 6, Y = 7.

groups; at 3×10^3 cells for group X ($P < 0.05$), and for groups Y, Z, and a ($P < 0.01$); at 10^4 cells for groups e, f, g, h, and i ($P < 0.01$), with one survivor at this dose (group g); and at 3×10^4 cells for groups l and m ($P < 0.01$).

DISCUSSION

Combined treatment of L1210 leukemia with ALK-NABS (0.8 ml) followed by CTX cured all mice in group I of experiment 1 (fig 1). In a previous study with the same pool of ALK-NABS, we found that when ALK-NABS was used alone, approximately one fourth the amount used in the present experiment was optimal (10). When this optimal dose of ALK-NABS (0.2 ml) was used in conjunction with CTX in experiment 2 of the present study, we obtained extension of lifespan but no permanent cures (group f, fig 2). One possible explanation for this disappointing result (fig 2) is that the optimal dose of ALK-NABS is higher when CTX is given in addition to ALK-NABS. However, this is not a likely explanation, since CTX is immunosuppressive (14) and ALK-NABS is either immunosuppressive or toxic (10); given *together* with CTX, an optimal dose for ALK-NABS *lower* than that when given alone would be expected.

A second and more likely explanation for the good results obtained in experiment 1, in contrast to the disappointing results in experiment 2, is that the L1210 cells used in experiment 1 had been transplanted for 37 generations since resurrection from frozen storage in liquid nitrogen at Arthur D. Little, Inc. All other experiments were performed with L1210 cells transplanted less than ten times since resurrection from frozen storage. I. Wodinsky of Arthur D. Little, Inc, has found that initial transplant generations of resurrected L1210 cells produce 100% deaths in BDF_1 mice for 10^3 cells injected ip, whereas at generation 15 there are usually some survivors at this dose (personal communication). Our experience with transplantation in DBA/2 mice, though far less extensive, is similar. In line with these data, the mean survival time of control groups A and B in experiment 1 was 13.1 days; in contrast, mean survival times of corresponding control groups that also received 10^5 L1210 cells in experiments 2 and 3 were 8.7 and 10.1 days respectively. While these results support the sug-

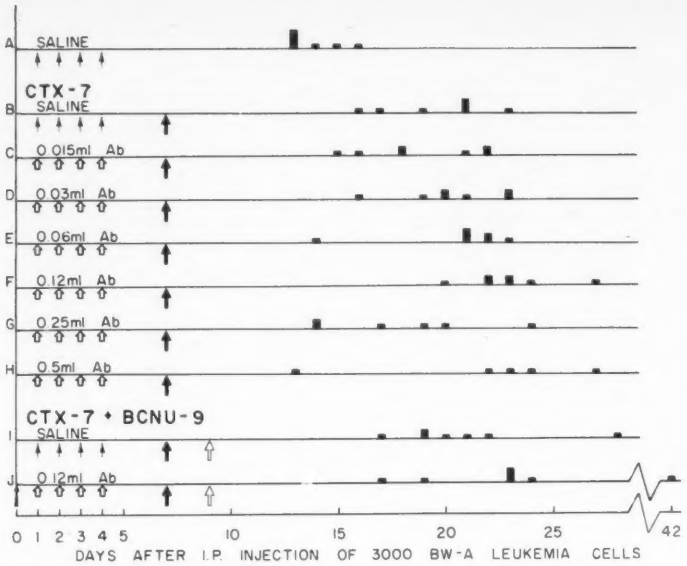

FIGURE 5.—Therapy experiment 5 with BW-A leukemia in AKR mice. Leukemia cells were given ip on Day 0 for all groups (A–J) identified by a letter above their horizontal line. All therapy injections were given ip at stated times (arrows) from Day 1 to 9. For each injection per day per 20.0-g mouse: saline (↓) = 1.0 ml isotonic NaCl, CTX (⇩) = 165 mg/kg cyclophosphamide, Ab (⇧) = stated amount of absorbed ALK-NABS prepared against BW-A leukemia, BCNU (⇩) = 8 mg/kg. Nos. of mice in different groups were A = 7, B = 7, C = 7, D = 7, E = 7, F = 7, G = 6, H = 5, I = 7, J = 7.

gestion that the immunogenicity of the L1210 cells used in experiment 1 was higher than that in later experiments, they are not definitive. Further experiments are presently in progress in our laboratory with several different L1210 lines to determine whether "other factors being equal, the effectiveness of immunotherapy runs parallel to the immunogenicity of the tumor in its host" (11).

A third possible explanation for poorer results in experiments done after experiment 1 is that certain antibodies lose titer with time unless kept at much lower temperatures than –10°C. This, however, is not a problem for rabbit antisera to mouse ascites tumors. We have previously found that the antitumor cytolytic activity of such an antiserum when stored at –10°C for almost 3 years did not change significantly (15).

The completely negative results for ALK-NABS immunotherapy in the AKR-BW-A model system require explanation. Since AKR lymphomas are almost exclusively thymic in origin (16), we might have been wiser to use anti-thymic rather than anti-splenic NABS for the preparation of the anti-BW-A ALK-NABS reagent. While this conclusion is also supported by the in vitro data of cytolytic potencies of this reagent (table 1), it seems impossible to predict the results of the in vivo therapy experiments (figs 1–6) from the in vitro data. This discrepancy emphasizes that results of immune cytolysis have little bearing on tumor biology or antigenic differences between tumor and host, which could be far more relevant regarding the outcome of immunotherapy.

On the positive side, low doses of ALK-NABS produced significant therapy of L1210 leukemia when used with CTX. Improvements in the in vitro absorption of such antisera, without introduction of additional toxicity, seem to be an essential next step. With further improvements, eventual use of this type of immunotherapy in man seems possible.

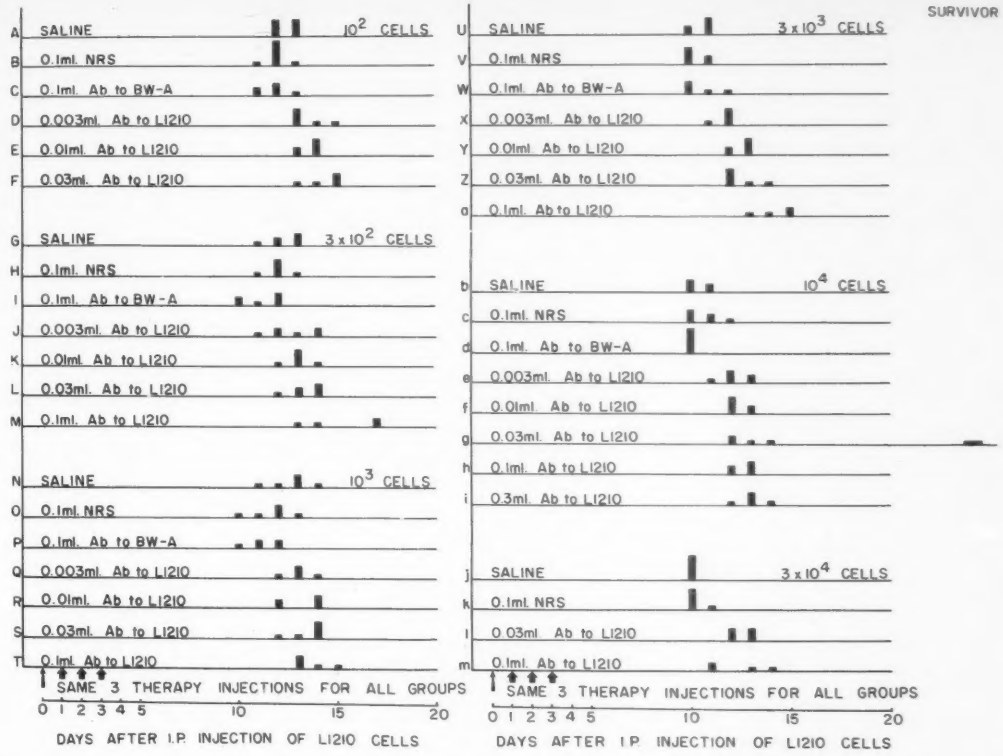
SURVIVOR
A SALINE
B 0.1ml. NRS
C 0.1ml. Ab to BW-A
D 0.003ml. Ab to L1210
E 0.01ml. Ab to L1210
F 0.03ml. Ab to L1210
10^2 CELLS
G SALINE
H 0.1ml. NRS
I 0.1ml. Ab to BW-A
J 0.003ml. Ab to L1210
K 0.01ml. Ab to L1210
L 0.03ml. Ab to L1210
M 0.1ml. Ab to L1210
3×10^2 CELLS
N SALINE
O 0.1ml. NRS
P 0.1ml. Ab to BW-A
Q 0.003ml. Ab to L1210
R 0.01ml. Ab to L1210
S 0.03ml. Ab to L1210
T 0.1ml. Ab to L1210
10^3 CELLS
U SALINE
V 0.1ml. NRS
W 0.1ml. Ab to BW-A
X 0.003ml. Ab to L1210
Y 0.01ml. Ab to L1210
Z 0.03ml. Ab to L1210
a 0.1ml. Ab to L1210
3×10^3 CELLS
b SALINE
c 0.1ml. NRS
d 0.1ml. Ab to BW-A
e 0.003ml. Ab to L1210
f 0.01ml. Ab to L1210
g 0.03ml. Ab to L1210
h 0.1ml. Ab to L1210
i 0.3ml. Ab to L1210
10^4 CELLS
j SALINE
k 0.1ml. NRS
l 0.03ml. Ab to L1210
m 0.1ml. Ab to L1210
3×10^4 CELLS
SAME 3 THERAPY INJECTIONS FOR ALL GROUPS
0 1 2 3 4 5 10 15 20
DAYS AFTER I.P. INJECTION OF L1210 CELLS
SAME 3 THERAPY INJECTIONS FOR ALL GROUPS
0 1 2 3 4 5 10 15 20
DAYS AFTER I.P. INJECTION OF L1210 CELLS

FIGURE 6.—Therapy experiment 6 with L1210 leukemia (transplant generation 9 following frozen storage) in DBA/2 mice. Stated Nos. of L1210 cells were given ip on Day 0 for all groups (A–m), identified by a letter above their horizontal line. All therapy injections were given ip on Days 1, 2, and 3 in 0.5 ml total volume. Stated volumes of the following reagents were injected per day per 20.0-g mouse: NRS, Ab to BW-A = absorbed ALK-NABS to BW-A, Ab to L1210 = absorbed ALK-NABS to L1210. Nos. of mice in different groups were: A = 8, B = 8, C = 6, D = 6, E = 6, F = 6, G = 6, H = 6, I = 6, J = 6, K = 6, L = 6, M = 4, N = 6, O = 6, P = 5, Q = 5, R = 5, S = 6, T = 5, U = 6, V = 6, W = 5, X = 5, Y = 6, Z = 6, a = 4, b = 5, c = 6, d = 6, e = 6, f = 6, g = 5, h = 5, i = 5, j = 6, k = 6, l = 6, m = 4. There was 1 survivor in group g.

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Specificity of Antisera Produced Against Bleomycin [1,2]

James E. Strong,[3,4] Alan Broughton,[3] and Stanley T. Crooke[3,5,*]

SUMMARY

The specificity of antisera produced in a goat or rabbit for use in bleomycin radioimmunoassay has been examined using competitive experiments employing several bleomycin analogs. These studies demonstrate that the antiserum distinguished alterations in both the nucleus and the terminal structure of the molecule. Removal of the terminal structure or addition of an aminosugar moiety in the bleomycin nucleus resulted in a significant decrease in immunoreactivity. Antisera produced in goat and rabbit hosts had different specificity for the terminal structure. The goat antisera demonstrated greater specificity for the terminal side chain than the rabbit antisera although this may not reflect a species-related event.

[Cancer Treat Rep 61:1509–1512, 1977]

Bleomycin is an antitumor antibiotic isolated from *Streptomyces verticillus* (1). It has been used clinically in the treatment of squamous cell carcinomas, lymphomas, and testicular carcinomas (2). Bleomycin probably induces its antitumor effects by producing single and double strand breaks in preformed DNA (3). The bleomycin employed clinically (Blenoxane, Bristol Laboratories, Inc) in the US is a mixture containing several closely related analogs (4). These components differ in their terminal side chain structures. At least 65% of the mixture in bleomycin is composed of species from the bleomycin A group, while the remaining 35% of the total weight is accounted for by members of the B group. The bleomycin A_2 and B_2 contents are 55%–70% and 25%–32% of the total weight, respectively, with a copper content of $< 0.1\%$ by weight (4). Numerous other analogs have been isolated or prepared semisynthetically (5).

Recently, a radioimmunoassay for bleomycin was developed that proved to be 100 times more sensitive than previously reported microbiologic assays (6). The two assays were shown to yield similar results (7) within the range of sensitivity for the microbiologic assay. Since antisera used in the radioimmunoassay were prepared with a bleomycin-bovine serum albumin conjugate, a study of the specificity of the antibodies raised in both rabbits and goats was undertaken.

MATERIALS AND METHODS

Chemicals.—Bleomycin sulfate (Blenoxane) and BU2231B were supplied by Bristol Laboratories. Bleomycinic acid, bleomycin A_2, B_2, A_5, B_1, iso-bleomycin A_2, and desamido bleomycin A_2 were supplied by Nippon-Kayaku, Tokyo, Japan. Bleomycinic acid, bleomycin A_2, B_2, and A_5 contained copper. However, BU2231B, iso-bleomycin A_2, bleomycin sulfate, and a second sample of bleomycin A_2 were copper free.

Immunization.—Two goats and three rabbits were immunized with a bleomycin-bovine serum albumin conjugate prepared using 1-ethyl-3-(dimethylaminopropyl)carbodiimide (6). Each animal received 0.5 mg of the conjugate emulsified in Freunds adjuvant (one part conjugate to two parts adjuvant) by im injection into each limb. This was followed by monthly booster injections of 0.5 mg of

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[2]Supported in part by a grant in aid from Bristol Laboratories (Syracuse, NY).

[3]University of Texas Health Science Center, Houston (J. E. Strong and A. Broughton); and Department of Medical Research, Bristol Laboratories, Syracuse, NY, and Department of Pharmacology, Baylor College of Medicine, Houston, Tex (S. T. Crooke).

[4]*Present address:* Department of Pharmacology, Baylor College of Medicine, Houston, Tex.

[5]We wish to acknowledge the excellent technical assistance of James A. Knight and also the invaluable help of Henry Carwile in the production of goat antisera. The excellent typographic assistance of Ms. Julie Durantini is also appreciated.

[*]*Reprint requests to:* Stanley T. Crooke, MD, Department of Research and Development, Bristol Laboratories, Thompson Rd, Syracuse, NY 13201.

TABLE 1.—Bleomycin analog immunoreactivity with rabbit antisera*

Bleomycin	Mean concentration (pmols)	SD (pmols)	% cross-reactivity
Sulfate	3.45	±0.11	100
A_2	2.99	±0.22	115.4
B_2	4.63	±0.17	74.5
A_5	29.50	±5.14	11.7
Iso-A_2	13.70	±0.29	25.2
Acid	> 1000.00	—	< 1.0
BU2231B	> 1000.00	—	< 1.0
Desamido A_2	8.26	0.51	44.6
B_1	214.2	15.9	1.6

*Indicated quantity of bleomycin analog was required to produce a 50% inhibition of ^{125}I-labeled bleomycin sulfate bound to antibody. Results are expressed as the mean concentration, in picomols, of the bleomycin analog determined in 5 separate analyses.

TABLE 2.—Bleomycin analog immunoreactivity with goat antisera*

Bleomycin	Mean concentration (pmols)	SD (pmols)	% cross-reactivity
Sulfate	2.36	±0.26	100
A_2	2.69	±0.59	87.7
B_2	35.3	±45.2	6.7
A_5	> 1000.0	—	< 1.0
Iso-A_2	8.33	±2.0	28.3
Acid	> 1000.0	—	< 1.0
BU2231B	> 1000.0	—	< 1.0
Desamido A_2	4.94	±1.76	47.8
B_1	> 1000.0	—	< 1.0

*Indicated quantity of bleomycin analog was required to produce a 50% inhibition of ^{125}I-labeled bleomycin sulfate bound to antibody. Results are expressed as the mean concentration, in picomols, of the bleomycin analog determined in 5 separate analyses.

conjugate into either the fore or hind limbs. The antisera obtained from the rabbit and goat yielding the highest titer (1:10,000 and 1:20,000, respectively) were used for the radioimmunoassay.

Immunoassay.—Competitive antibody-binding experiments for bleomycin analogs were performed by adding the indicated concentration of the analog to a solution containing antisera plus iodine-125 labeled bleomycin sulfate and measuring the amount of labeled drug bound by antibody as previously described (6). The immunoreactivity was expressed as the amount of bleomycin analog required to produce 50% inhibition of the ^{125}I-bleomycin bound.

RESULTS

Immunoreactivity of bleomycin analogs relative to bleomycin sulfate for the rabbit antisera is depicted in table 1. Bleomycin analogs that were in the immunization mixture cross-reacted with ^{125}I-labeled bleomycin sulfate. Iso-bleomycin A_2 and desamido bleomycin, which are not normally contained in bleomycin sulfate, also competed for antibody binding. However, bleomycinic acid and BU2231B did not significantly compete for antibody binding. Bleomycin B_1 competed with low efficiency for antibody binding.

Immunoreactivity of the goat antisera for bleomycin analogs is depicted in table 2. There were significant differences in the specificity of this antisera and the rabbit antisera. The cross-reactivity of bleomycin B_2 was much less than the rabbit antisera. Bleomycin A_5 and B_1 also did not compete significantly for antibody binding. However, the goat antisera reacted similarly to the rabbit antisera with desamido bleomycin A_2 and iso-bleomycin A_2.

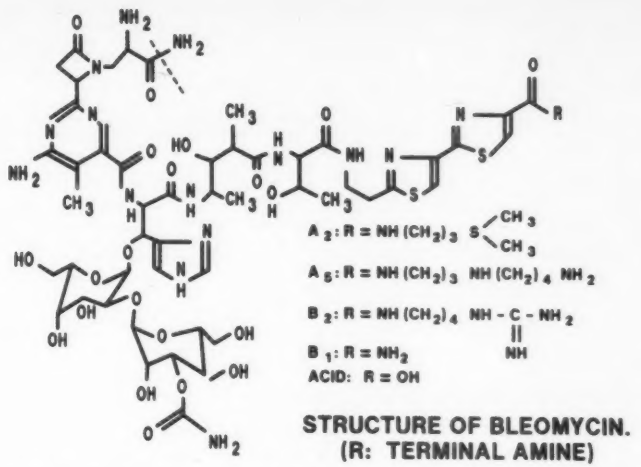

FIGURE 1.—Structure of bleomycin analogs. * = site of the carbamic acid group in iso-bleomycin A_2; — — — = site deaminated to produce desamido bleomycin A_2.

Figure 1 shows the general composition of bleomycin and the terminal groups for bleomycin A_2, A_5, B_2, and B_1. Bleomycinic acid does not contain a terminal group. Iso-bleomycin A_2 has the same structure as bleomycin A_2, except the carbamic acid group of the mannose sugar is found on the 2-carbon instead of the 3-carbon position. BU2231B is a new bleomycin analog (2) having a peptide portion containing four of the amino acids found in bleomycin (fig 2). The primary difference between BU2231B and bleomycin appears to be the substitution of a methyl-4-amino-4,6-dideoxy-L-talopyranoside in the bleomycin nucleus.

DISCUSSION

Competition of bleomycin analogs with ^{125}I-labeled bleomycin sulfate for antibody recognition provides evidence for the antibody recognition sites of antisera prepared using bleomycin sulfate. Lack of the terminal structure or alteration in the bleomycinic nucleus effectively eliminated antibody recognition.

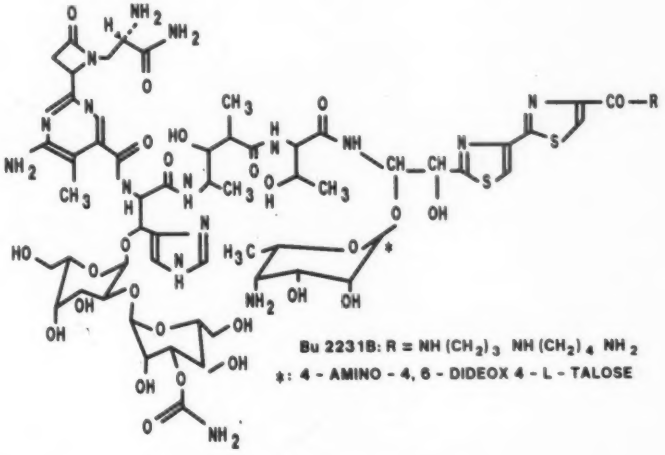

FIGURE 2.—Structure of BU2231B.

Also, migration of the carbamic acid on the mannose moiety producing iso-bleomycin A_2 caused a fourfold decrease in efficiency of competition for both the rabbit and goat antisera. Since the immunodominant groups of a hapten are usually those distal to the site of conjugation with the carrier protein (8), the site of conjugation was probably not near the mannose sugar or the terminal group. Thus, two possible sites for carbodiimide-mediated bleomycin conjugation to bovine serum albumin could have been either the amine group on the B-aminoalanine or the amine group attached to the pyrimidine moiety.

The goat antisera appeared to have greater sensitivity for changes in the terminal structure of bleomycin than the rabbit antisera. This specificity was most evident with the difference in reactivity for bleomycin B_2. Bleomycin A_5 also reacted significantly differently in the two analyses and cross-reacted much more with the rabbit antisera than with the goat antisera. Thus, use of the goat antisera for radioimmunoassay would measure primarily bleomycin A_2, whereas rabbit antisera would measure both bleomycin A_2 and B_2.

The presence of copper had no apparent effect on cross-reactivity since copper-chelated bleomycin A_2 behaved similarly to copper-free bleomycin A_2.

Desamido bleomycin is reportedly a metabolite produced by the action of a bleomycin-inactivating enzyme upon bleomycin B_2 (9). Both the goat and rabbit antisera show significant affinity for this compound. The presence of the desamido bleomycin in biologic specimens could interfere with measurement of the parent compound by radioimmunoassay. Such interference may become significant when the unmetabolized drug is present at low concentrations following administration of bleomycin. Serum specimens from patients in renal failure have been analyzed for bleomycin concentration by both radioimmunoassay and microbiologic assay (7) and there has been a high correlation between the two methods ($r = 0.987$). Thus, if metabolites were present in these specimens, they did not significantly alter the correlation between immunoreactivity and biologic activity. However, since the radioimmunoassay is approximately 100-fold more sensitive than the microbiologic assay, interference by metabolites may yet be significant at concentrations lower than the microbiologic assay sensitivity.

Note: The authors will be pleased to supply small quantities of bleomycin radioimmunoassay antiserum to qualified investigators.

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Cellular Response to Treatment With 4-(3-(2-Chloroethyl)-3-nitrosoureido)-*cis*-cyclohexanecarboxylic Acid, a Water-Soluble Nitrosourea Derivative[1,2,3]

Benjamin Drewinko,* Catherine Green, and Ti Li Loo[4]

SUMMARY

The lethal effects of 4-(3-(2-chloroethyl)-3-nitrosoureido)-*cis*-cyclohexanecarboxylic acid (*cis*-acid), a water-soluble nitrosourea derivative, were investigated on a human lymphoma cell line. The survival of asynchronous cells exposed to increasing concentrations of the drug was characterized by a threshold exponential curve (D_o = 20 μg/ml; D_q = 20 μg/ml, 1 hour) similar to that of other nitrosourea derivatives. *cis*-Acid exerted its main killing effect on cells in early S and in late G_2 phase. Cells in mid S and early G_1 phase were tenfold more resistant. Changes in survival response as a function of cell cycle stage were reflected primarily by changes in the extent of the shoulder region of the survival curve. In contrast to other nitrosoureas, the lethal effectiveness of *cis*-acid in solution was stable and the drug could sterilize large numbers of cells in short periods of time. Another important major difference observed for *cis*-acid with respect to classic nitrosourea derivatives was the capacity of treated cells to recover from sublethal and potentially lethal damage. Our studies have shown that *cis*-acid is as effective in killing cultured human lymphoma cells as other nitrosoureas, but possibly with a mechanism different from that of these compounds. The major shortcoming noted for *cis*-acid, namely the capacity of treated cells to recover from drug-induced damage, is offset by the relatively long stability of its killing effect. This, and the fact that *cis*-acid can be administered in an aqueous solution, make this agent an appealing compound for clinical trials.

[Cancer Treat Rep 61:1513–1518, 1977]

4-(3-(2-Chloroethyl)-3-nitrosoureido)-*cis*-cyclohexanecarboxylic acid (*cis*-acid) is a new nitrosourea derivative synthesized at the Kettering-Meyer Laboratory, Southern Research Institute (Birmingham, Ala) (1). Since its log octanol-water partition coefficient is –1.14, the compound is quite soluble in water and, in contrast to other nitrosoureas, it can be administered in an aqueous solvent (2). *cis*-Acid was active against L1210 leukemia, early and advanced Lewis lung carcinoma (2,3), B16 melanoma, C3H mammary tumor, and colon tumor No. 26 (Montgomery JA, personal communication) and compared favorably with its congener methyl-CCNU (meCCNU).

The objective of this study was to investigate the lethal effects of *cis*-acid on cultured human lymphoma cells in order to compare its activity with that of three previously studied nitrosourea derivatives (4). Dose-response curves, capacity to recover from drug-induced damage, and cell cycle stage-dependent activity were defined. *cis*-Acid compared very closely to the above-mentioned nitrosoureas

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[3]4-(3-(2-Chloroethyl)-3-nitrosoureido)-*cis*-cyclohexanecarboxylic acid: NSC-153174.

[4]Departments of Laboratory Medicine (B. Drewinko and C. Green) and Developmental Therapeutics (T. L. Loo), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Benjamin Drewinko, MD, PhD, Department of Laboratory Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

although its killing effectiveness in solution was much more stable.

MATERIALS AND METHODS

Drugs.—*cis*-Acid was obtained in crystalline form from the Kettering-Meyer Laboratory through the courtesy of Dr. J. A. Montgomery. It was easily dissolved in neutral 0.9% NaCl solution immediately before each experiment. Serial drug concentrations were prepared in growth medium.

Cell line.—A human immunoglobulin-producing cell line (T_1 cells) derived from the tumorous lymph node of a patient with lymphocytic lymphoma was used (5). Monolayer cultures were maintained in Ham's F-10 medium supplemented with 20% fetal calf serum, vitamins, glutamine, and antibiotics. Under these conditions, the recently determined cell cycle time by the pulse-labeled mitosis method was 31 hours, the pre-DNA synthesis period (G_1) was 15 hours, the DNA synthesis period (S) was 10 hours, and the post-DNA synthesis period (G_2) was 6 hours (6,7). The calculated mitotic time was 0.54 hour.

Cell synchronization.—Synchronized S phase cells were obtained by a single treatment with 3 mM thymidine for 24 hours as previously described (8). The degree of synchrony was monitored in replicate cultures by 30-minute pulse labeling with 1 microcurie of ^{3}H-deoxycytidine/ml to determine the percentage of cells in S phase and by scoring the mitotic index (MI), and by DNA histograms obtained by pulse cytophotometry as previously reported (8). In all radioautography procedures, a 50% solution of Ilford K5 emulsion (Polysciences, Inc, Warrington, Pa) in distilled water was used. Labeled cells were identified by the presence of five or more grains overlying the nucleus. At the end of the synchronization procedure (0 hour) 85%–90% of the cells were in S phase. This level was maintained for the next 6 hours after which the proportion of S phase cells declined rapidly; at 13 hours about 78% of the cells were in G_2 phase. A mitotic peak ranging from 5% to 12% occurred between 15 and 17 hours and cells subsequently entered G_1 phase composing 84% of the population at 28 hours.

Dose-response survival of asynchronous populations.—Stock cultures of T_1 cells were harvested by previously reported standard procedures (9) and counted with the aid of an electronic particle counter (Coulter Counter Model ZBI, Coulter Electronics, Inc, Hialeah, Fla). Cell suspension aliquots were seeded into 60-mm petri dishes (0.5×10^6 cells/dish). The cells were incubated at 37°C in a 5% CO_2 atmosphere in air for about 48 hours to achieve exponential growth. The medium was discarded and the cells were exposed to increasing drug concentrations for exactly 1 hour at 37°C. The drug was decanted and the cells were washed twice with Hank's balanced salt solution, harvested as a monodispersed suspension, and counted. Known aliquots were dispensed into 60-mm petri dishes so that 50–100 colonies would appear after 21 days' incubation in a 5% CO_2 humidified atmosphere at 37°C. The colonies were stained with 0.5% crystal violet in 95% ethanol. Viability was defined as the ability of single cells to give rise to a colony of ≥ 50 cells. In each experiment, the plating efficiency of at least six control cultures was assessed simultaneously. Control cultures consisted of cells treated in exactly the same manner as the test cells but without receiving drug. The survival fractions for the different drug concentrations were normalized with respect to the individual controls for each experiment. In this series of experiments, the plating efficiency ranged from 15% to 40%. All experiments were repeated at least twice with triplicate samples for each drug concentration and exposure time.

Kinetics of cell kill.—Cell survival as a function of duration of treatment was investigated by exposing replicate cultures simultaneously to a single dose of drug. Petri dishes containing 0.5×10^6 cells in asynchronous exponential growth were incubated at 0 time with a single concentration of drug (5, 10, or 50 μg/ml in different experiments). Replicate cultures were harvested at regular intervals for 24 hours and processed for colony-formation assays described above. Plating efficiency controls were obtained at regular intervals. To demonstrate the stability of the lethal activity of *cis*-acid, a separate control system was instituted. This consisted of dishes also containing 0.5×10^6 cells which were kept growing without adding drug throughout the experiment. Every time a time-point dish was harvested, its supernatant medium was transferred to the control dishes after discarding their drug-free medium. The latter was now incubated for 1 hour at 37°C and survival was assessed as usual.

Cell cycle stage-dependent cell survival.—Age-dependent survival was investigated by incubating synchronized cells with increasing concentrations of drug at regular intervals throughout the cell cycle. Plating efficiency controls were obtained at each time point.

Recovery.—To investigate recovery from drug-induced damage, cells were exposed to fractionated doses of the integral dose. Integral dose was defined as the product of the concentration times the time ($C \times t$) of incubation (10,11). Thus, asynchronous exponentially growing cells received the total integral dose in two equal separate exposures spaced in

time (50 μg/ml × 15 minutes each), and their survival was compared to that resulting from cells receiving the total dose at one time (100 μg/ml × 15 minutes). Controls obtained at regular intervals consisted of cells receiving (*a*) one half the integral dose (50 μg/ml × 15 minutes); (*b*) the total integral dose (100 μg/ml × 15 minutes); and (*c*) the total integral dose where only the time parameter was changed (50 μg/ml × 30 minutes). In addition, in order to define recovery from potentially lethal damage, cells treated with one concentration of *cis*-acid (50 μg/ml) were washed and reincubated at 37°C with spent medium for 8 hours before harvesting and plating for colony formation.

RESULTS

The survival curve of T_1 cells exposed to increasing concentrations of *cis*-acid for 1 hour at 37°C was of the threshold exponential type C pattern described for other nitrosourea derivatives (4). After a moderately large shoulder region (D_q = 20 μg/ml, 1 hour), survival decreased exponentially as a function of escalating concentrations (D_0 = 20 μg/ml, 1 hour) (fig 1).[5] These results are within the range of values determined for other nitrosourea derivatives on the same cell line (4).

Cells exposed to a single concentration of *cis*-acid (5, 10, or 50 μg/ml) were completely sterilized after a 4-hour treatment. Immediately after exposure to the drug, cells were washed and harvested, and inocula with known cell titers were seeded into 60-mm petri dishes. No colonies were observed (except for some small abortive ones) after 21 days of incubation, even when the initial inoculum had contained 10^5 cells/dish. Therefore, cell killing as a function of exposure interval could not be studied for longer periods because the survival of the treated cells was below the sensitivity of our assay by 4 hours.

The drug-containing supernatants of cells treated with *cis*-acid for periods ranging up to 24 hours were used to treat control, previously unexposed, T_1 cells for 1 hour at 37°C. The survival of these cells was always decreased (10%–15%) in comparison to that observed for cells treated for 1 hour with freshly prepared drug, but the results were not statistically significant. The decrease in survival was greatest for 24-hour-old supernatants.

Cell age-dependent survival investigated on synchronized cells revealed fluctuations in sensitivity.

[5] D_q = quasi-threshold dose equal to the intercept with the abscissa of the exponential part of survival curve; D_0 = mean lethal dose equal to the concentration required to reduce survival by 63% on exponential part of survival curve.

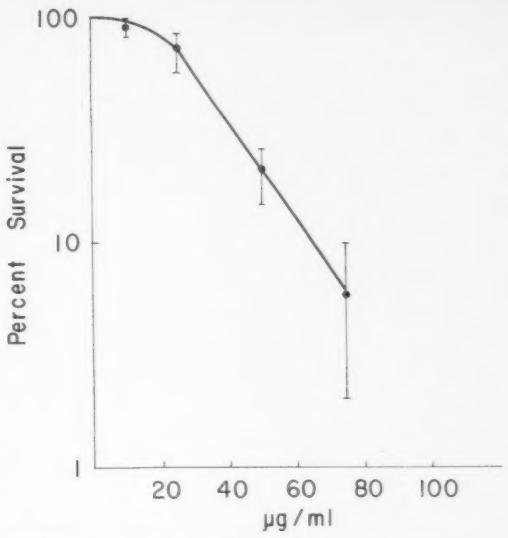

FIGURE 1.—Dose-dependent survival response to T_1 cells incubated with increasing concentrations of *cis*-acid (D_0 = 20 μg/ml, and D_q = 20 μg/ml, 1 hr). Points are mean values of 5 independent experiments each with 3 replicates/dose point. Bars represent SEs.

The most sensitive stages of the cycle were early S and late G_2 phase. Cells in mid to late S and G_1 phases were about tenfold more resistant (fig 2). These changes in sensitivity were reflected primarily by modifications in the extent of the shoulder region (fig 3). The D_0 values of early S and late G_2 phase cells were similar.

In contrast to other nitrosourea derivatives, cells exposed to *cis*-acid demonstrated a remarkable capacity to recover from sublethal damage. Cells treated with fractionated doses showed increased survival in comparison to those receiving the total dose at once (fig 4). Additionally, cells which were harvested 8 hours after termination of treatment, and which had been maintained during that interval growing in spent medium, revealed 70% greater survival than those plated immediately after treatment.

DISCUSSION

BCNU, CCNU, and meCCNU are clinically useful cancer chemotherapeutic agents (12,13). Although their in vivo activity is qualitatively similar (14), their chemical and physicochemical properties (15) are not identical, and they display differential lethal effects at the cellular level (4). All three nitrosourea derivatives share a common structural feature,

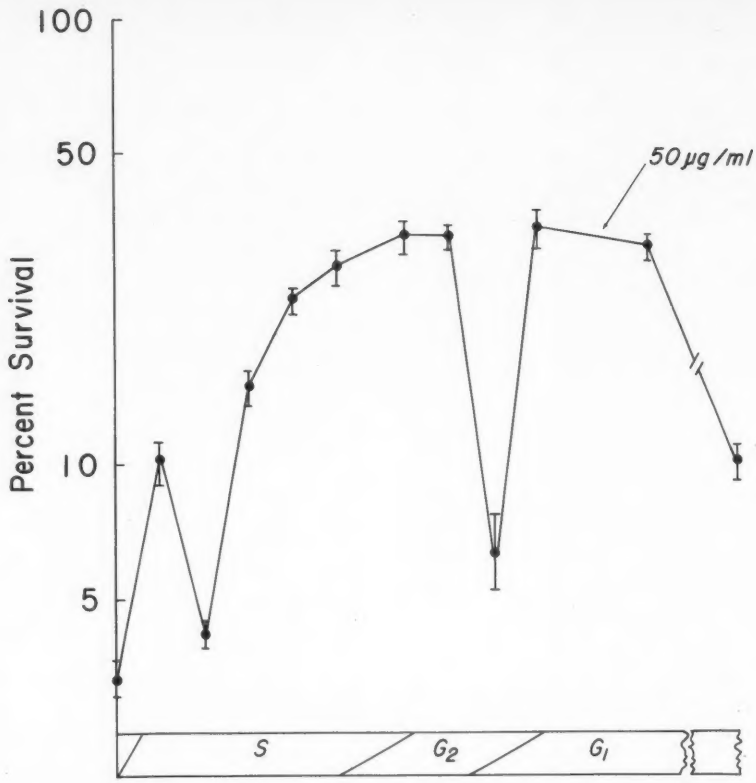

FIGURE 2.—Survival of synchronized T_1 cells exposed to 50 µg/ml of *cis*-acid for 1 hr at selected points of the cell cycle. Points are average values of 2 separate experiments each with 3 replicates/time point.

namely, a 2-chloroethyl moiety on the nitrogen that bears the nitroso group; however, the substituents on the other nitrogen are different (16). Thus, CCNU and meCCNU are derivatives of cyclohexane. MeCCNU demonstrated the greatest activity against Lewis lung carcinoma (17) which prompted the synthesis and evaluation of a series of meCCNU analogs for the treatment of solid tumors (1). The highest activity was observed for compounds with a substituent on the cyclohexane ring in the *trans* position to the ureido nitrogen; the *cis* isomers were far less active (16). *cis*-Acid was an exception in that it was at least as active as its *trans* counterpart. In addition, *cis*-acid has another desirable property distinctly different from the lipid-soluble nitrosoureas, namely, its solubility in aqueous solutions. Hence, it offers the advantage of ready formulation in injectable form for clinical use. Although, admittedly, high lipid solubility enables a drug to negotiate plasma membranes with ease, this property may be disadvantageous in that lipophilic drugs tend to remain in the membrane failing to translocate into the cytoplasm. Potentially, *cis*-acid is sufficiently lipid-soluble to easily penetrate the cell membrane but not too soluble to remain there indefinitely.

In this study, we investigated the lethal effects of *cis*-acid on a cultured human lymphoma cell line in order to compare this agent to other nitrosourea derivatives previously studied in this line (4). Asynchronous exponentially growing T_1 cells exposed to increasing concentrations of *cis*-acid showed a survival response qualitatively and quantitatively similar to that resulting from the treatment with BCNU, CCNU, and meCCNU. However, in contrast to these compounds, the lethal activity of *cis*-acid was stable in solution and the drug was capable of rapidly sterilizing the cell population in short periods of time. In this respect, *cis*-acid approximated the effect of meCCNU which, in solution, exerted

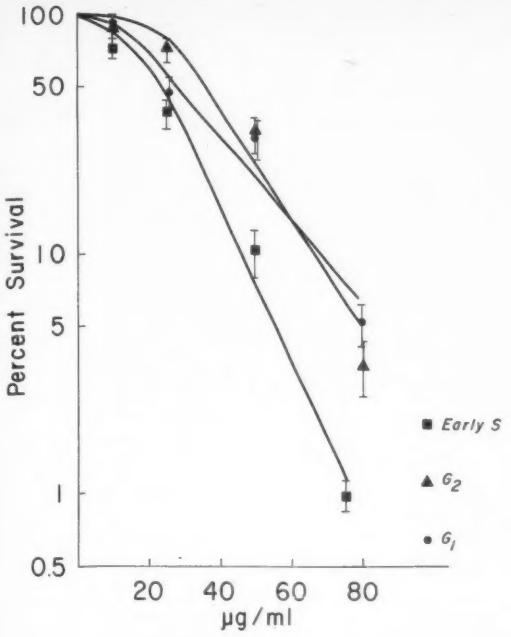

FIGURE 3.—Dose-dependent survival of synchronized T_1 cells exposed to increasing concentrations of *cis*-acid for 1 hr at selected points of the cell cycle. Early S phase cells were obtained 2 hrs after the release of thymidine treatment. G_2 and G_1 phase cells correspond to cells selected 13 and 24 hrs following removal of the synchronizing agent. Data points are mean values from 2 independent experiments each with 3 replicate dishes/concentration point. Bars represent SEs.

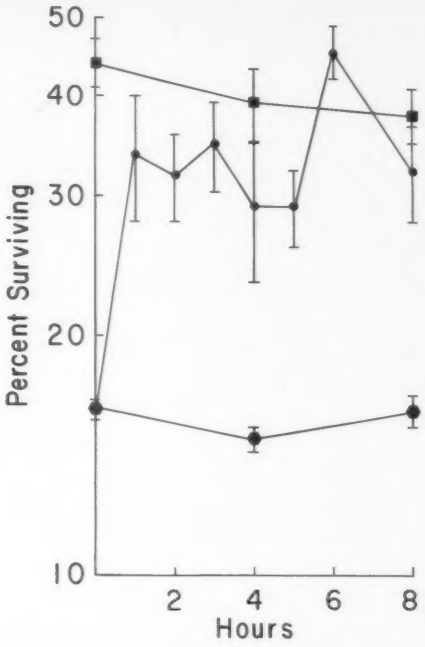

FIGURE 4.—Survival of T_1 cells after split-dose treatment with *cis*-acid. Upper curve (squares) demonstrates the survival of cells exposed to 50 μg/ml for 15 mins at the times indicated by the points. Lower curve (hexagons) indicates the survival of cells exposed to 100 μg/ml for 15 mins at the times indicated by the points. The middle curve (circles) represents the survival of cells exposed to 50 μg/ml for 15 mins at time 0, washed, reincubated with fresh medium, and exposed to an additional 50 μg/ml of *cis*-acid for 15 mins at the times indicated by the points. Cells incubated with 50 μg/ml for 15 mins at 0 hr, reincubated with spent medium, and harvested 8 hrs later had a survival of 69.4% ± 4.7%.

considerable lethality on T_1 cells for periods up to 24 hours (4). The lethal activity of nitrosourea derivatives is believed to be mediated by highly reactive breakdown products with relatively short half-lives (18,19). It is possible that the nature of the alkylating and carbamylating decomposition compounds of *cis*-acid differ from those noted for classic nitrosoureas, thus conferring the observed longevity of its killing efficiency in solution. In this context, it is interesting that, even though the lethal activity of 24-hour-old drug-containing supernatants did not differ statistically from that observed for freshly prepared drug, the killing efficiency was always greater by 10%–15%, suggesting that *cis*-acid had undergone biochemical transformations that rendered the solution slightly more effective than freshly prepared drug. Furthermore, it is noteworthy that T_1 cells were able to recover from *cis*-acid-induced damage while no significant recovery was observed for the same cells treated with BCNU, CCNU, or meCCNU (4). Failure to recover from the killing effect of the latter nitrosoureas could be related to at least one degradation product, 2-chloroethyl isocyanate, known to inhibit repair of DNA single-strand breaks (20). Since recovery was present after treatment with *cis*-acid, it would appear that its decomposition does not yield a similar by-product.

When *cis*-acid was incubated with synchronized cells, survival fluctuated as a function of position in the cycle in a manner similar to that observed for other nitrosourea derivatives. Yet, the pattern of the survival curve was different from that of the other compounds. Qualitatively, the effect of *cis*-acid on early S phase cells was similar to all three major nitrosourea derivatives, but, quantitatively, the results approximated those obtained with

CCNU and meCCNU. In contrast, the marked killing effect in G_2 phase observed with *cis*-acid had been noted only for cells treated with BCNU.

Another major important difference of *cis*-acid with respect to the classic nitrosoureas was the capacity of the treated cells to recover from sublethal and potentially lethal damage. Apparently, the primary action of *cis*-acid at the level of the target molecule is not an irreversible injury and can be repaired by cellular activity. Thus, the differential effect of these compounds may reside in the fact that, while *cis*-acid affects only the target site, the other derivatives injure both target site and molecular repair mechanisms.

Our studies have shown that *cis*-acid is as effective in killing cultured human cells as other nitrosoureas, but possibly with a mechanism different from that of these compounds. The major drawback noted for *cis*-acid, namely capacity to recover from sublethal damage, is offset by its relatively long biologic stability. This and the fact that *cis*-acid is water-soluble make this agent an appealing compound for clinical trials.

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Preparation and Antitumor Evaluation of Water-Soluble Derivatives of Dichloro(1,2-diaminocyclohexane)platinum(II) [1,2]

Paul Schwartz,[3] Sandra J. Meischen,[3] Glen R. Gale,[3,4,*] Loretta M. Atkins,[3,4] Alayne B. Smith,[3,4] and Ernest M. Walker, Jr[5]

SUMMARY

The structure of the antitumor agent, dichloro(1,2-diaminocyclohexane)platinum(II) (NSC-194814), was modified by replacing the chlorides with organic or inorganic anions. Eighteen new platinum complexes were so isolated and their antitumor properties against the L1210 leukemia in C57BL/6 × DBA/2 mice were evaluated. Most of the complexes were readily soluble in water and some had enhanced antitumor activity compared to the parent dichloro complex. In addition, increased solubility with retention of significant antitumor activity was obtained by oxidizing the parent dichloroplatinum(II) complex with halogen or peroxide to give two platinum(IV) complexes. Some previously reported platinum complexes with phosphorus, selenium, or tellurium electron donor ligands were also synthesized and assessed for antitumor action, but these did not show appreciable activity.

[Cancer Treat Rep 61:1519–1525, 1977]

An earlier report from this laboratory described the antitumor activity of a variety of *cis*-amineplatinum congeners of *cis*-dichlorodiammineplatinum(II) (NSC-119875) against the L1210 leukemia in C57BL/6 × DBA/2 (BDF_1) mice (1). Of 46 complexes tested, dichloro(1,2-diaminocyclohexane)platinum(II) (NSC-194814) was the most active. On an equimolar dose basis it was appreciably more active than NSC-119875 which is presently being used in clinical trials. However, the very low aqueous solubility of the diaminocyclohexane complex precludes its use in phase I clinical trials. Increased solubility was obtained by substitution of the chlorides with other organic and inorganic anions, and certain ones of these were water soluble and retained antitumor activity. In addition, all active congeners were synergistic when used in combination chemotherapy with cyclophosphamide (1,2).

We now report the syntheses and antileukemic properties of 20 new platinum(II) and platinum(IV) complexes. We also assessed the antitumor activity of two previously reported platinum amine complexes as well as some platinum complexes with non-nitrogen electron donor ligands.

MATERIALS AND METHODS

Chemical

Dinitrato(1,2-diaminocyclohexane)platinum(II) (NSC-239851), sulfato(1,2-diaminocyclohexane)-platinum(II) (NSC-250427), and NSC-194814 were prepared as described in a previous report (1). New platinum(II) complexes were prepared by one of the four following routes; representative preparations are illustrated in figure 1.

Route I: reaction of NSC-194814 with the silver salt of the ligand. Silver chloride precipitated and the supernatant solution was evaporated to dryness to yield the product.

Route II: reaction of NSC-239851 with the potassium

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[3]VA Hospital, Charleston, SC.

[4]Department of Pharmacology, Medical University of South Carolina, Charleston.

[5]Departments of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston.

**Reprint requests to:* Dr. Glen R. Gale, Research Service, VA Hospital, 109 Bee St, Charleston, SC 29403.

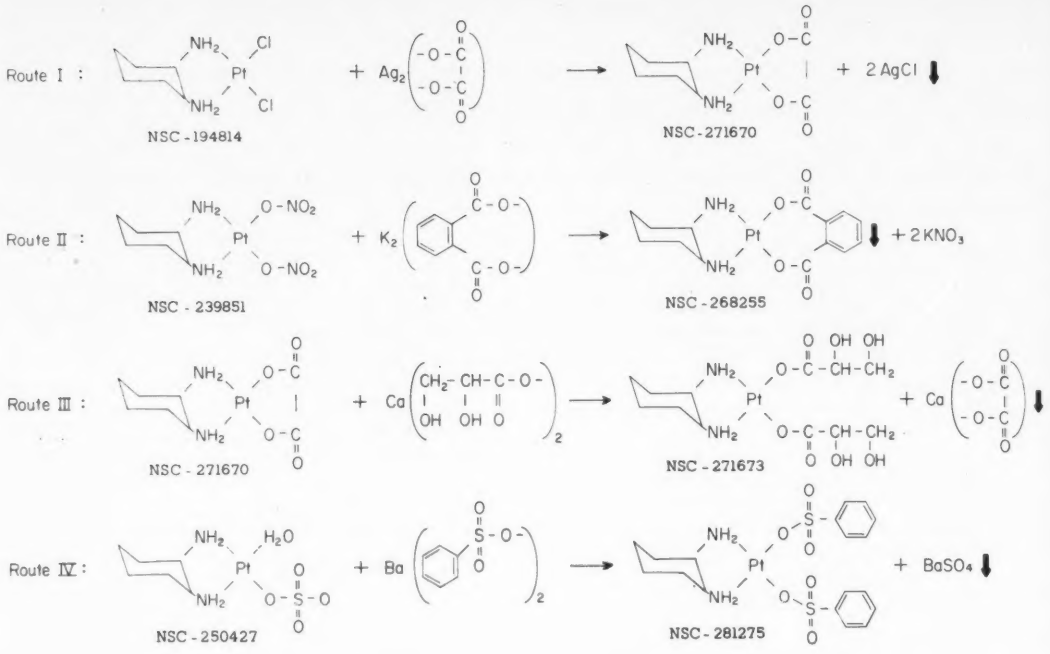

FIGURE 1.—Representative routes for the preparation of new platinum(II) derivatives. The bold vertical arrow in each case represents precipitation of the compound.

salt of the ligand. This method was useful when the desired product was of low aqueous solubility. The complex precipitated and was washed with ethanol and dried in vacuo.

Route III: reaction of oxalato(1,2-diaminocyclohexane)platinum(II) (NSC-271670) with the calcium salt of the ligand. Calcium oxalate precipitated and the supernatant solution was evaporated to yield the product.

Route IV: reaction of NSC-250427 with the barium salt of the ligand. Barium sulfate precipitated and the supernatant solution was evaporated to yield the product.

Table 1 lists all the platinum(II) derivatives of NSC-194814 which were prepared, NSC numbers, routes of preparation (*see* above), % yields, and results of platinum elemental analyses (Galbraith Laboratories, Knoxville, Tenn).

Two platinum(IV) complexes were also prepared. Tetrachloro(1,2-diaminocyclohexane)platinum(IV) (NSC-276017) was prepared by oxidizing the dichloro complex with 30% hydrogen peroxide. Concentrated hydrochloric acid was added dropwise to the resultant suspension and a bright yellow solution was formed. The solution was evaporated to dryness and the residue was recrystallized from hot water to give bright yellow crystals.

trans-Dibromo-*cis*-dichloro(1,2-diaminocyclohexane)platinum(IV) (NSC-271678) was prepared by the addition of bromine to a suspension of NSC-194814 in chloroform. An orange solid formed after the mixture was stirred for 0.5 hour. It was collected and washed with cold water and ether and then air-dried.

Seven other platinum complexes were prepared and tested for antitumor activity. Six of these had been reported previously in the literature but had not been assessed for biologic activity. In addition, the sulfate derivative of one of these complexes [*cis*-dichlorobis(dimethylselenide)platinum(II) (NSC-271675)] was prepared by a method analogous to route I (above) and was designated sulfatobis(dimethylselenide)platinum(II) hydrate (NSC-281278). These are shown in table 2.

Biologic

Antileukemic activity was evaluated as in the previous paper (1). The BDF_1 and DBA/2 male mice

TABLE 1.—Derivatives of NSC-194814

NSC No.	Derivative	Route of preparation	% yield	% platinum	
				Theoretic	Found
258376	Hydroxonitrato(1,2-diaminocyclohexane)platinum(II)	*	26	50.16	49.22
263158	Di(methanesulfonato)(1,2-diaminocyclohexane)platinum(II)†	I	90	39.06	38.26
263160	Methylmalonato(1,2-diaminocyclohexane)platinum(II)	II	56	45.87	45.62
264414	Di(*p*-toluenesulfonato)(1,2-diaminocyclohexane)platinum(II) hydrate	I	82	29.13	29.31
265486	Tartrato(1,2-diaminocyclohexane)platinum(II) 2½ hydrate	I	36	38.83	38.94
265487	Orthophosphato(1,2-diaminocyclohexane)platinum(II)‡	I	58	46.09	45.85
265488	Acetylacetonato(1,2-diaminocyclohexane)platinum(II) nitrate§	II	31	41.47	41.41
268252	Dihydroxo(1,2-diaminocyclohexane)platinum(II) dihydrate	IV	36	51.43	51.54
268253	Dipyruvato(1,2-diaminocyclohexane)platinum(II) hydrate	I	79	38.90	38.85
268254	1,2-Cyclohexanedicarboxylato(1,2-diaminocyclohexane)platinum(II)	II	34	40.68	40.61
268255	Phthalato(1,2-diaminocyclohexane)platinum(II) hydrate	II	21	39.61	39.69
271670	Oxalato(1,2-diaminocyclohexane)platinum(II)	I	18	49.10	49.23
271671	Glycerophosphato(1,2-diaminocyclohexane)platinum(II) hydrate	III	85	39.22	39.12
271672	Di(gluconato)(1,2-diaminocyclohexane)platinum(II) trihydrate	III	94	25.26	26.20
271673	Di(glycerato)(1,2-diaminocyclohexane)platinum(II)	III	85	37.56	37.35
271674	(4-Carboxyphthalato)(1,2-diaminocyclohexane)platinum(II)	II	60	37.70	37.33
271677	(4-Hydroxyphthalato)(1,2-diaminocyclohexane)platinum(II)	II	36	39.27	39.86
271678	*trans*-Dibromo-*cis*-dichloro(1,2-diaminocyclohexane)platinum(IV)	*See* text	65	36.12	36.27
276017	Tetrachloro(1,2-diaminocyclohexane)platinum(IV)	*See* text	80	43.25	43.09
281275	Di(benzenesulfonato)(1,2-diaminocyclohexane)platinum(II)	IV	85	30.48	30.30

*Preparation adapted from ref 3.
†Hygroscopic.
‡Blue complex.
§Positively charged complex.

TABLE 2.—Platinum complexes with non-leaving ligands other than 1,2-diaminocyclohexane

NSC No.	Complex	Ref No.	Electron donor atom
263161	Dichloro(ethylenediamine-*N,N'*-diacetic acid)platinum(II)	4	N
264413	Dichloro(1,3-diaminopropane-2-ol)platinum(II)	5	N
271675	*cis*-Dichlorobis(dimethylselenide)platinum(II)	6	Se
271676	*cis*-Dichlorobis(dimethyltelluride)platinum(II)	6	Te
276018	*cis*-Dichlorobis(diphenylselenide)platinum(II)	7	Se
276019	Dichloro(1,2-bisdiphenylphosphinoethane)platinum(II)	8	P
281278	Sulfatobis(dimethylselenide)platinum(II) hydrate*		Se

*New compound: 50% yield; platinum analysis, theoretic (35.78%) and found (35.27%).

were provided by the Mammalian Genetics and Animal Production Section, Division of Cancer Treatment, National Cancer Institute. BDF_1 recipients for testing were given 10^6 L1210 cells ip from DBA/2 donors on Day 0. Mice were treated ip with a single injection on Day 1 only. Activity was measured as % increase in lifespan (%ILS) compared to control untreated mice. Toxic levels were approximated by observing the effects (weight changes, death) of each compound on tumor-free Swiss-Webster female mice.

RESULTS AND DISCUSSION

The %ILS values obtained with each new compound at several dose levels are listed in table 3. Approximate toxic levels (LD50) and water solubilities are also shown. For comparison, the optimum %ILS values obtained in this laboratory with NSC-119875 and NSC-194814 under the described conditions are also listed. Several complexes conferred ILS values in excess of 200%, and occasional cures (> 60-day survival) were observed.

It is noteworthy that replacing the chlorides of NSC-194814 with nitrate (NSC-239851) (1), sulfate (NSC-250427) (1), hydroxide [dihydroxo(1,2-diaminocyclohexane)platinum(II) dihydrate (NSC-268252)], or orthophosphate [orthophosphato(1,2-diaminocyclohexane)platinum(II) nitrate (NSC-265487)] gave rise to very active compounds. They all produced > 100% ILS values at dose levels considerably below the approximate LD50 doses. These inorganic anions dissociate at a fast rate and are generally considered highly labile leaving groups. Cleare and Hoeschele (9) correlated the leaving rates of ligands with the antitumor activity of a number of platinum complexes and found that the most active complexes contained ligands of moderate lability, approximating that of chloride. Ligands with fast leaving rates gave rise to highly toxic complexes, while those with slow leaving rates gave rise to inactive, nontoxic complexes. For example, the dinitrato derivative of NSC-119875 was a highly toxic complex which caused convulsions and death several hours after administration of 7 mg/kg to Swiss-Webster mice. It was ineffective as an antitumor agent in a dose range from 2 to 20 mg/kg (9). On the other hand, NSC-239851, the dinitrato analog of NSC-194814, produced a 164% ILS at 4 mg/kg and had an LD50 of approximately 10 mg/kg (1). Apparently the diaminocyclohexane moiety reduces the toxicity while increasing the antitumor activity of complexes with highly reactive ligands.

In addition to the above-mentioned compounds, we prepared effective antitumor complexes with anions of monocarboxylic, dicarboxylic, sulfonic, and phosphoric acids. The dicarboxylic acid dianions (oxalate, malonates, and phthalates) chelate to the central platinum(II) cation and form neutral complexes. While phthalato(1,2-diaminocyclohexane)platinum(II) (NSC-268255) is very insoluble, 4-carboxyphthalato(1,2-diaminocyclohexane)platinum(II) (NSC-271674) readily dissolves in 1% sodium bicarbonate solution via formation of the sodium carboxylate. This characteristic would be of considerable benefit in potential phase I clinical trials since iv administration would thereby be facilitated.

Another chelating agent, the acetylacetonate monoanion, forms a positively charged platinum complex (acetylacetonato(1,2-diaminocyclohexane)platinum(II) nitrate) (NSC-265488). It was the least active of all the derivatives of NSC-194814 which were prepared and tested. This is consistent with the low activity found with other positively charged complexes (9).

Rosenberg et al (10) showed that the antitumor activity of tetrachlorodiammineplatinum(IV) was comparable to that of NSC-119875. However, relatively little work on the biologic activity of additional platinum(IV) complexes has been reported. In a recent review of platinum complexes possessing

TABLE 3.—Antileukemic activity of platinum complexes*

NSC No.†	Dose (mg/kg)	%ILS‡	Vehicle§	Approximate LD50¶ (mg/kg)	Approximate aqueous solubility (mg/ml)
119875	8	119	1	9	1
194814	5	139	2	25	< 0.1
258376	1	64			
	3.75	73	3	20	15
	5	111			
	7.5	112			
	10	144			
	15	92			
263158	1	69	3	15	> 30, extremely hygroscopic
	2	93			
	2.5	108			
	4	112			
	5	116			
	7.5	132			
	8	136			
	10	132			
263160	50	67	4	> 100	0.3
	100	88			
263161	25	27	1	100	3
	50	34			
264413	2.5	49	2	12	0.5
	5	64			
	10	19			
264414	2.5	74	3	15	3
	5	88			
	10	83			
265486	12.5	92	4	60	1
	25	121			
	50	129			
265487	6.25	88	3	60	> 30
	12.5	89			
	25	126			
	50	135			
265488	10	27	3	60	5
	20	36			
	40	25			
	60	14 (NS)			
268252	1	65	3	15	> 30
	2	80			
	4	116			
	6	141			
	8	167			
268253	5	43	3	50	15
	10	63			
	15	72			
	25	83			
	30	91			
268254	50	61	4	> 100	< 0.1
	100	91			
268255	50	144	4	> 100	< 0.1
	100	146			
271670	1	30	3	30	1
	2.5	59			
	5	90			
	10	120			
	15	132			
	20	131			

TABLE 3.—Antileukemic activity of platinum complexes*—Continued

NSC No.†	Dose (mg/kg)	%ILS‡	Vehicle§	Approximate LD50¶ (mg/kg)	Approximate aqueous solubility (mg/ml)
271671	5	61	3	60	1
	10	80			
	20	100			
	30	130			
	40	147			
	50	42			
271672	5	40	3	> 100	1
	10	60			
	20	93			
	25	108			
	40	121			
	50	163			
	60	229(1)**			
271673	2.5	44	3	40	1
	5	49			
	10	73			
	15	99			
	20	99			
271674	5	70	5	75	2
	10	118			
	20	119			
	25	139			
	40	232			
	50	288(1)			
	60	174(1)			
271675	15	24	2	50	< 0.1
	30	22			
271676	15	0	2	50	< 0.1
	30	2(NS)			
271677	15	157	4	75	< 0.1
	30	207			
	60	261(1)			
271678	3	110	3	12	2
	6	149			
	9	159			
276017	2	74	1	10	2
	4	159			
	8	226(1)			
276018	25	0	2	> 100	< 0.1
	50	1(NS)			
	100	3(NS)			
276019	100	2(NS)	2	> 200	< 0.1
	200	0			
281275	5	124	3	20	1
	7.5	163			
	10	239(1)			
281278	25	17	3	100	5
	50	22			
	75	18			

*Mice received 10^6 L1210 cells on Day 0; treatment was on Day 1 only.

†Names of all new compounds are given in tables 1 and 2.

‡Unless followed by NS (not significant), each %ILS value was significant at the ≥ 99% confidence level.

§1 = clear solution in 0.9% NaCl; 2 = suspension in 0.9% NaCl; 3 = clear solution in 5% glucose solution; 4 = suspension in 5% glucose solution; 5 = clear solution in 1% $NaHCO_3$ solution.

¶Estimated from data obtained with Swiss-Webster mice as well as by inspection of data in this table showing decreasing %ILS values at higher doses with certain compounds.

**Nos. in parentheses indicate the No. of cures (> 60-day survivors) in each group of 8 treated mice.

antitumor activity, Leh and Wolf (11) stated that the central platinum atom should be at 2^+ oxidation state. We found, however, that the dibromodichloro (NSC-271678) and the tetrachloro (NSC-276017) platinum(IV) complexes had activity at least equal to that of NSC-194814 along with improved water solubilities. Platinum(IV) complexes provide an important class of potential antitumor agents and should not be ignored when designing new platinum-containing compounds.

There has been much interest and controversy over the role of selenium in biochemical processes. Some investigators have reported that selenium is oncogenic (12), while others dispute that claim and maintain that selenium has antineoplastic properties (13). We were interested in determining what effect a selenium donor ligand would have on the antitumor activity of platinum complexes. To date, all reported active platinum complexes contain nitrogen donor ligands as non-leaving groups. We found marginal activity (24% ILS) with NSC-271675 at 15 mg/kg. The analogous diphenylselenide complex [*cis*-dichlorobis(diphenylselenide)platinum(II) (NSC-276018)] and the dimethyltelluride complex [*cis*-dichlorobis(dimethyltelluride)platinum(II) (NSC-271676)] showed no activity under our test conditions. The inactivity of these complexes may be due to steric factors as it was shown that the activity of platinum complexes with secondary or tertiary amine ligands is substantially reduced compared with complexes with primary amine ligands (9). We feel that the meager amount of data which has accrued on complexes with non-nitrogen donor ligands is insufficient to rule them out entirely in the search for new antitumor agents; additional investigations in this area are clearly warranted.

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Phase II Study of Combined Vincristine, Adriamycin, Cyclophosphamide, and Methotrexate With Citrovorum Factor Rescue in Metastatic Breast Cancer [1]

Willy Mattsson,* Ake Arwidi, Finn von Eyben, and Claes-Ebbe Lindholm [2]

SUMMARY

Fifty patients with metastatic breast cancer were treated with 8-day courses of vincristine (1 mg iv, Day 1), adriamycin (50 mg/m² iv, Day 1), cyclophosphamide (100 mg/m² orally, Days 1–8), methotrexate (200 mg iv by 3-hour infusion, Day 8), and citrovorum factor rescue (15 mg im 12, 18, and 24 hours after methotrexate, Day 8) at 3–4-week intervals. Forty-two patients had previously received treatment with hormones and 17 patients had received chemotherapy. Fifteen patients achieved a complete remission (CR) and 24 patients a partial remission (PR). There was a significant correlation between the response and the number of metastatic organs ($0.01 < P < 0.02$). The response rate was roughly uniform irrespective of the organ predominantly involved ($0.2 < P < 0.3$). The remission duration was significantly longer for the patients with CR compared with that for the patients with PR ($0.001 < P < 0.01$). The patients who achieved CRs and PRs survived significantly longer than the patients with no change and progressive disease ($P < 0.001$). The toxic effects of the treatment were acceptable and no drug-related deaths occurred.

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Combination chemotherapy for metastatic breast cancer has a well-documented beneficial effect. An objective response can be expected in 50%–80% of all cases (1–5). The response rate of skeletal metastases is often lower (6,7) as is that of treatment of relapses after another type of chemotherapy (8,9).

This paper summarizes the results of combined treatment with vincristine, adriamycin, cyclophosphamide, and methotrexate with citrovorum factor rescue (VACM regimen) with special reference to the effect on skeletal metastases and on relapses in patients previously treated with cyclophosphamide, methotrexate, 5-fluorouracil (5-FU), and prednisone (CMFP), according to Canellos et al (10).

MATERIALS AND METHODS

Fifty patients with advanced breast cancer were accepted in a phase II study. The criteria for inclusion were: measurable lesions, leukocyte counts $\geq 2.0 \times 10^9$/liter, platelet counts $\geq 100 \times 10^9$/liter, sulfobromophthalein (BSP) retention test $\leq 25\%$, and age ≤ 75 years.

The median age was 55 years (range, 32–74 years). In 26 patients the disease had been diagnosed before menopause. All together, the patients had previously been treated for metastases with 102 therapeutic measures (median, 2.0). Forty-two patients had received hormones, 27 radiation, and 17 cytotoxic chemotherapy (table 1), and 16 had undergone surgery. The previous hormonal therapy was as follows. All 26 premenopausal patients had been oophorectomized. When progressive disease occurred, 16 of them had been treated successively with both androgens and tamoxifen and seven patients with androgens alone. Seven of the postmenopausal patients had successively received both estrogens and tamoxifen and 12 had received tamoxifen therapy. The median number of metastatic organs was three (table 2). The predominant metastatic site was soft tissue in six patients, skeleton in 12, and viscera in 32 (table 3).

The following 1-week regimen was used. On Day

[1] Received Apr 22, 1977; revised July 18, 1977; accepted July 28, 1977.

[2] Department of Radiotherapy, General Hospital, Malmö, Sweden.

*Reprint requests to: Willy Mattsson, MD, Department of Radiotherapy, General Hospital, 214 01 Malmö, Sweden.

TABLE 1.—Results of VACM regimen according to previous chemotherapy

Previous chemotherapy	No. of patients	Results of VACM regimen				Duration of remission (mos)	
		CR	PR	No change	Progressive disease	Median	Range
CMFP (ref 10)	14	1	9	2	2	13+	2–22+
5-FU liver infusion	3	1	2			14	7–16+
Total	17	2	11	2	2		

TABLE 2.—Results in relation to No. of metastatic organs*

No. of metastatic organs	No. of responses				
	CR	PR	No change	Progressive disease	Total
1	4	3	0	0	7
2	6	5	1	0	12
3	1	9	1	0	11
≥4	4	7	4	5	20
Total	15	24	6	5	50

*There is a significant correlation between No. of metastatic organs and response (Kruskal-Wallis 1-way analysis of variance by ranks, $0.01 < P < 0.02$).

TABLE 3.—Results in relation to predominant metastatic organs

Predominant organ	No. of responses				
	CR	PR	No change	Progressive disease	Total
Soft tissue	3	2	1	0	6
Visceral	9	14	4	5	32
Osseous	3	8	1	0	12
Total	15	24	6	5	50

1, patients were treated iv with 1 mg of vincristine and 50 mg/m² of adriamycin. From Days 1 to 8 inclusive the patients were given cyclophosphamide, 100 mg/m² orally. On Day 8, the patients received methotrexate, 200 mg by drip infusion for 3 hours, with subsequent citrovorum factor rescue, 15 mg im at 12, 18, and 24 hours after the end of the infusion of methotrexate. The patients received such a course on six occasions at 3-week intervals, after which they were given the adriamycin regimen every fourth week until they had received a total dose of, at most, 500 mg/m². In patients in remission treatment with the same regimen was continued except that adriamycin was replaced by 5-FU, 500 mg/m² by iv drip infusion during 6 hours. The courses were then repeated every sixth week as long as the patients were in remission. This time table was carefully observed. In the event of myelosuppression, the dose was adjusted according to a special modification scale (table 4). The dose of adriamycin was adjusted according to the BSP retention test: BSP ≤ 9%, 100% of the calculated dose; BSP 10%–15%, 75% of the calculated dose; and BSP 16%–25%, 50% of the calculated dose.

The results of treatment were assessed from physical examinations and assessment of Karnofsky's performance index before every course. In all patients roentgenograms of the lung and skeleton as well as isotope scans of the liver and skeleton were regularly performed at intervals of ≤ 3 months. All metastatic lesions of the lung, skeleton, and liver were examined with suitable roentgenograms and/or isotope scans before every second course up to ten courses in 38 of the patients.

TABLE 4.—Dose-modification scale*

Leukocytes* (× 10⁹/liter)	Platelets* (× 10⁹/liter)	% of calculated dose of— Vincristine	Adriamycin	Cyclophosphamide	Methotrexate
3.9–3.0	125–100	100	75	75	100
2.9–2.0	99–75	100	50	50	75
≤ 1.9	≤ 74	Prolongation of interval			

*Pretreatment values.

Determinations of the hemoglobin level and leukocyte and platelet counts were made every week, and before at least every other course the laboratory studies included determination of differential cell counts, serum iron, total iron binding capacity, electrolytic status, creatinine, and liver function tests. Electrophoresis, ECG, and isotope scan of the heart were examined every 3 months.

The criteria used for remission were those recommended by Hayward et al (11); complete remission (CR) is the disappearance of all known disease. In the case of lytic bone metastases these must be shown radiologically to have calcified. Partial remission (PR) is a ≥ 50% decrease in measurable lesions and objective improvement in evaluable but nonmeasurable lesions; no new lesions can occur. No change is recorded when the lesions are unchanged (< 50% decrease or < 25% increase in the sites of measurable lesions). Progressive disease is recorded when some lesions regress while others progress or new lesions appear, or when some or all lesions progress and/or new lesions appear.

With special regard to metastatic lesions of the liver our criteria were as follows. All metastases of the liver were verified by cold areas of liver scans. In 12 patients the pathologic liver scan was verified by histologic examination of biopsy specimens or cytologic examination of fine needle aspirations. A CR was defined as a normalized liver scan further documented with a negative cytologic examination of specimens from fine needle biopsy directed towards the previous metastatic region. A PR was defined as a decrease of ≥ 50% of the cold area as visualized by liver scan. Otherwise the metastases of the liver were regarded as nonresponding. The duration of remission was calculated from the date of an objective response to a relapse and the survival from the beginning of treatment to death.

RESULTS

Thirty-nine (78%) of the 50 patients achieved objective response (15 CRs, 24 PRs). In six patients the disease became stationary (no change). Only five patients did not respond. In the complete responders the median number of metastatic organs was two compared with three in the patients in whom the remission was partial and four and five, respectively, in patients with no change and progressive disease. The response, analyzed by Kruskal-Wallis one-way analysis of variance by ranks, showed a significant relation to the number of metastatic organs ($0.01 < P < 0.02$) (table 2).

Five of six patients with predominant metastases in the soft tissue and 11 of 12 with predominant skeletal metastases had a remission. Of 32 patients with predominant visceral disease a remission was observed in 23, including nine in whom the remission was complete (table 3).

So far, the median duration of remission is 15+ months (range, 9+–25+ months) and 11+ months (range, 4–22+ months) for CR and PR, respectively. Twelve of the 15 complete responders as well as nine of the 24 partial responders are still in remission. The duration of remission was significantly longer for complete responders than for partial responders ($0.001 < P < 0.01$, log-rank test, fig 1).

Previously, seven of 14 patients had achieved an objective remission by CMFP therapy according to Canellos et al (10). All of these seven patients and three nonresponding patients showed an objective regression to this VACM regimen. Three patients previously achieved a PR with 5-FU given by intra-arterial infusion of the liver. When relapsing, an objective remission was obtained by VACM therapy. The median duration of remission was 13+ months, which did not differ from that in the other patients in this series (table 1).

When metastases of the skin and of the lymph nodes responded, they always did so within, respectively, three and four courses of treatment. Metastatic lesions of the lung and of the pleura responded later, but all responses occurred within five courses. Judging from skeletal surveys and liver scans, an objective response of metastatic lesions of the skeleton and liver usually required more courses, and objective responses were obtained after up to ten courses of treatment. The

TABLE 5.—Objective response according to involved organ

Involved organ	No. of patients with involved organ	No. of patients with objective response in involved organ	% response	No. of courses required to achieve objective response							
				1	2	3	4	5	6	8	10
				No. of patients							
Skin	25	22	88	7	9	6					
Lymph node	29	25	86	6	8	8	3				
Lung	28	21	74	—	5	10	4	2			
Pleura	12	10	—	2	2	3	2	1			
Liver	16	12	75	—	—	2	2	3	—	3	2
Bone	32	24	75	—	1	—	6	2	4	5	6

rate of response was roughly uniform irrespective of the organs involved ($0.2 < P < 0.3$, Kruskal-Wallis one-way analysis of variance by ranks) (table 5). The responders (CR + PR) survived significantly longer than the nonresponders (no change + progressive disease) ($P < 0.001$, log-rank test, fig 2).

Before therapy, the Karnofsky performance index showed a median of 70 points (range, 40–90 points). Thirty-seven of 39 responders improved their performance status. The median improvement was 30 points (range, 0–60 points). Twelve initially bedridden patients could lead an almost ordinary life during remissions. Fourteen of the responding patients returned to their usual work. Furthermore, the quality of life improved for four of six patients in whom the disease showed no change.

The side effects were not serious. No life-threatening toxic effects, sepsis, bleeding, nephrotoxicity, or diarrhea was noted. Alopecia, which occurred in all the patients, was reversible. Oral mucositis was observed in three patients on six occasions. In these patients citrovorum factor rescue was then prolonged to six courses of 15 mg im with 6-hour intervals, and no further mucositis occurred. In one patient cardiotoxicity was suspected, but the symptoms promptly disappeared after treatment with digoxine and diuretics. In 12 patients vincristine was withdrawn because of neurotoxicity after a total dose of 4–24 mg. Toxic effects of clinical importance (nausea and vomiting requiring hospital care or nadir values of leukocytes $\leq 1.9 \times 10^9$/liter and platelets $\leq 50 \times 10^9$/liter) were noted in 11 patients; toxic effects of minor clinical importance (transient nausea for >6 hours although treated with antiemetics or nadir values of leukocytes 2.9–2.0 $\times 10^9$/liter and platelets 74–51 $\times 10^9$/liter) were noted in 11; and toxic effects of no clinical importance (transient nausea easily treated with antiemetics or nadir values of leukocytes 3.9–3.0 $\times 10^9$/liter and platelets 100–75 $\times 10^9$/liter) were noted in 23. In five patients no side effects were observed (table 6).

DISCUSSION

The response rate (78%) and the median duration of remission (11+–15+ months) were about the same as in other series on record (1–10), but the effect on skeletal metastases (75%) was better as well as the rate of response to this regimen when used as a second-line treatment. A better effect on the skeletal metastases cannot be due solely to differences in the criteria for remission. One possi-

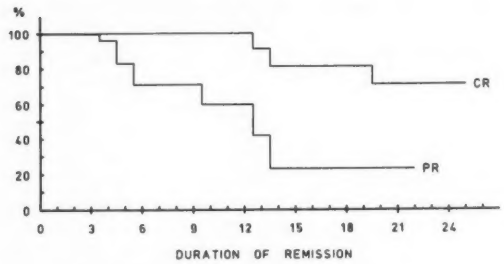

FIGURE 1.—Duration of remission for patients with CR was significantly longer than that for patients with PR ($0.001 < P < 0.01$, log-rank test).

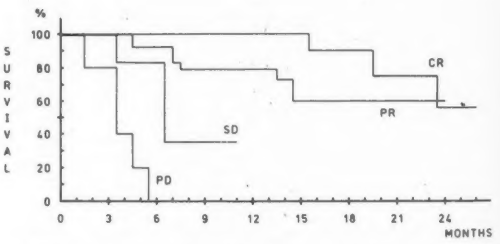

FIGURE 2.—Patients with CR or PR survived significantly longer than patients with no change or progressive disease ($P < 0.001$, log-rank test).

TABLE 6.—Toxicity

Grade of toxicity*	No. of patients
None	5
No clinical importance	23
Minor clinical importance	11
Clinical importance	11
Life-threatening	—

*Definitions in text.

ble reason is the prolongation of therapy, since the objective response of the skeleton, as judged from skeletal surveys and/or isotope scans, was mostly not demonstrable until after several courses of treatment. This explanation is strengthened by the fact that after two to seven courses of therapy the skeletal surveys of six of the later responders showed "progressive" lytic metastases. As these patients were then free from symptoms and other measurable lesions had regressed, the treatment was continued. Later, the skeletal surveys showed an objective response. In three patients the lytic metastatic lesions became sclerotic and then totally disappeared. Thus, the difference in response rate compared with that in other series on record might be explained either by the assumption that this VACM regimen was especially suitable for treating skeletal metastases or, more probably, that earlier investigators had stopped treatment too early.

The median duration of remission did not vary with the organs which were involved. However, we found a higher incidence of objective remission when fewer organs were involved. This might reflect the tumor load (increased tumor load implies a lower growth fraction and thereby fewer tumor cells available for the cytotoxic effects). Another possibility could be a higher incidence of tumor clones of different chemotherapy resistance with increased number of metastatic organs.

Ten of 14 patients who did not respond to or relapsed after previous CMFP therapy according to Canellos et al (10) achieved objective remission by this VACM regimen. About the same median duration of remission was obtained in these patients compared with the other patients who had not previously been treated with cytostatics; this occurred in spite of the fact that two of the drugs in the CMFP regimen are similar. This might be explained by the synergism between adriamycin and cyclophosphamide and vincristine, respectively (12,13), as well as the recent report that adriamycin combined with methotrexate can overcome experimental methotrexate resistance (14). Thus, this VACM regimen has probably no cross-resistance to CMFP therapy. The number of complete responders in this subgroup (one of 14) was slightly less than in the other patients (14 of 36). This might be due to the greater number of organs involved with metastases in this subgroup (median, four; range, two to seven).

This combination chemotherapy is well tolerated and can be used in outpatients. About 80% of the patients have achieved an objective, fairly long remission with improvement in quality of life irrespective of the sites of the metastases.

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Chemotherapy for Mustard-Resistant Ovarian Adenocarcinoma: A Randomized Trial of CCNU and Methyl-CCNU [1,2]

G. Omura,* P. DiSaia, J. Blessing, R. Boronow, M. Hreshchyshyn, and R. Park [3]

SUMMARY

In view of the need for drugs which have antitumor activity in alkylating agent-resistant ovarian cancer or which might be more potent than standard agents, a randomized trial of two nitrosoureas, CCNU and methyl-CCNU, has been conducted. Of 31 evaluable patients who received at least one dose of CCNU (100 mg/m² orally every 6 weeks), there were only two patients who experienced even transient improvement; 18 of these patients had two or more doses over a trial period of ≥ 12 weeks. Of 26 evaluable patients who received at least one dose of methyl-CCNU (150 mg/m² orally every 6 weeks), there was one patient who experienced transient improvement; this group included 14 patients receiving at least two doses and whose trial lasted ≥ 12 weeks. All patients had been previously treated with mustard-type alkylating agents. None of the responses were clinically useful. Thus, women with ovarian adenocarcinoma who have a history of prior chemotherapy are not likely to benefit from treatment with these nitrosoureas.

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There is evidence that the nitrosoureas are not cross-resistant with mustard-type alkylating agents both in animal systems (1) and in some human cancers such as Hodgkin's disease (2). In view of the broad spectrum of action of the nitrosoureas in different types of cancer and in view of the need to identify agents other than mustard-type alkylating agents which might be useful in treating ovarian adenocarcinoma instead of or subsequent to mustard-type treatment, the GOG has conducted a clinical trial of the nitrosoureas. Since the relative activity of CCNU and methyl-CCNU was uncertain, a randomized trial was performed.

MATERIALS AND METHODS

Women with biopsy-proven ovarian adenocarcinoma (stage III and IV) whose disease was not amenable to further surgical treatment or radiotherapy and who had failed to respond to mustard-type alkylating agents such as melphalan, cyclophosphamide, or chlorambucil, were eligible for treatment if they had at least one easily measurable mass, had recovered from toxic effects of prior treatment, had an estimated survival of at least 3 months, and had given written informed consent for protocol treatment. Since eligible patients would have had prior chemotherapy with or without radiotherapy, the drug doses were selected in anticipa-

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[2]CCNU and methyl-CCNU were supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute (NCI), Bethesda, Md.

[3]This study was performed by the Gynecologic Oncology Group (GOG). The writing committee included *G. Omura* (University of Alabama in Birmingham); *P. DiSaia* (University of Southern California, Los Angeles); *J. Blessing* (Roswell Park Memorial Institute, Buffalo, NY); *R. Boronow* (University of Mississippi, Jackson); *M. Hreshchyshyn* (Buffalo General Hospital, NY); and *R. Park* (Walter Reed Army Medical Center, Washington, DC).

The following institutions also participated in this study: University of Alabama in Birmingham (grant CA-12484 from the NCI, National Institutes of Health, Department of Health, Education, and Welfare), Duke University (CA-12534), Hahnemann and Jefferson Medical Colleges (CA-12478), New York Medical College (CA-12480), Rush Medical School (CA-12485), Walter Reed Army Medical Center, Wayne State Medical Center (CA-12477), Mt. Sinai Hospital (NY), Buffalo General Hospital (CA-12476), Emory University, University of Southern California (CA-13641), University of Mississippi (CA-13633), University of California at Los Angeles (CA-13630), and The George Washington University (CA-13446).

**Reprint requests to:* G. Omura, MD, University of Alabama in Birmingham, Medical Center, University Station, Birmingham, Ala 35294.

tion of limited tolerance for myelotoxic drugs. CCNU was given at 100 mg/m², or methyl-CCNU was given at 150 mg/m², every 6 weeks orally on an empty stomach if the wbc count was $>4000/mm^3$ and the platelet count was $>150,000/mm^3$.

Blood cell counts were to be obtained every 2 weeks. Dose reductions were allowed for subsequent treatments if the blood cell count nadirs were excessively low or the time to recovery was delayed. An adequate trial was defined as at least two courses with at least a 4-week followup after the second dose. Random assignment of treatment was conducted by the closed envelope technique; the sealed envelopes were provided by the GOG Statistical Office.

A complete regression was defined as disappearance of all measurable tumor and relevant symptoms. A partial regression was defined as >50% reduction in the total tumor area. Improvement was defined as between 25% and 50% reduction in the total tumor area; static disease was a <25% change over baseline measurements; progression was a >25% increase in the total tumor area in one measurable lesion or appearance of a new lesion. Any change in tumor measurement must have persisted for at least 4 weeks to be of significance.

RESULTS

Response

Of 82 patients who were entered into the study, 25 were ineligible or inevaluable. Of 39 women randomized to CCNU, eight were excluded (one, lost to followup; one, previous breast cancer; one, insufficient data; one, medication error; three, uncertain primary; and one, no prior chemotherapy). Of 43 randomized to methyl-CCNU, 17 were excluded (three, uncertain primary; one, wrong primary; four, intercurrent disease preventing study; two, medication error; four, insufficient data; one, previous breast cancer; one, refusal of treatment; and one, no prior chemotherapy). Thus, of the patients with ovarian adenocarcinoma (table 1), there were 31 evaluable patients who received CCNU; 13 had one dose, 11 had two doses, four had three doses, two had four doses, and one had seven doses. Only two showed "improvement" (serous; mixed epithelial). Of 26 patients who received methyl-CCNU, 12 had one dose, nine had two doses, four had three doses, and one had four doses; one showed improvement (mucinous). The age range for all patients was 28–76 years, with a range of 39–76 years for CCNU and 28–68 years for methyl-CCNU.

TABLE 1.—Response vs No. of courses (doses) of chemotherapy

No. of courses	Results of treatment	
	CCNU	Methyl-CCNU
1	1 death	
	1 static disease	
	11 progressive disease	12 progressive disease
2	1 improvement	1 improvement
	5 static disease	3 static disease
	5 progressive disease	5 progressive disease
≥ 3	1 improvement	
	2 static disease	2 static disease
	4 progressive disease	3 progressive disease

The groups appeared comparable. If the patients receiving only one dose are excluded (the protocol defined an adequate trial as two doses), there were 18 patients who received two or more doses of CCNU; seven were static, nine progressed, and two showed improvement. Of the 14 patients who had two or more doses of methyl-CCNU, five were static, eight progressed, and one showed improvement. There was no indication that improvement or static disease was clinically useful.

Table 2 shows the histologic subtypes of ovarian cancer treated with each agent.

Toxicity

Of 31 patients receiving CCNU, ten were not evaluable for toxicity because of lack of data. Of those who were evaluable for toxicity (21 patients), one patient died from pancytopenia (drug-related death), three patients had wbc counts $<2000/mm^3$, and seven patients had platelet count nadirs $<75,000/mm^3$ including two who had counts $<25,000/mm^3$. Of 26 patients receiving methyl-CCNU, 11 were not evaluable for toxicity. Three of 15 patients had a wbc count nadir $<2000/mm^3$ and

TABLE 2.—Evaluable adenocarcinomas

Histologic subtype	No. of evaluable adenocarcinomas	
	CCNU	Methyl-CCNU
Serous	20	16
Mucinous	3	4
Clear cell	1	0
Endometrioid	1	2
Undifferentiated	2	1
Mixed epithelial	1	1
Other	3	2
Total	31	26

one had a platelet count nadir < 75,000/mm³. Nausea was common with both drugs but was not dose-limiting. Other toxic effects were not documented.

The drug death which occurred was in a 47-year-old woman with a history of whole-abdominal radiation plus multiple-drug therapy. Her renal function was normal but her alkaline phosphatase was markedly elevated. Seventeen days after the first dose, she became comatose and died with a platelet count of 2000/mm³ and wbc count of 1000/mm³. No autopsy was done but an intracranial hemorrhage was probably the cause of death.

DISCUSSION

Mustard-type alkylating agents have a good therapeutic index in ovarian adenocarcinoma with 30%–50% of the patients showing at least a partial response (3,4) to agents such as melphalan and chlorambucil, which are usually well tolerated with minimal toxic effects. New agents must either be shown to have greater activity as primary chemotherapy, or to be active in patients who have failed to respond to mustards, or to have less toxicity before clinical utility in this disease is established. Cruz et al (5) and Sasaki et al (6) suggest that one of the nitrosoureas is useful in ovarian cancer. Sasaki et al reported that seven of 18 of their patients with advanced ovarian adenocarcinoma responded to CCNU given at 100–130 mg/m² every 6 weeks; however, most of their patients had had no prior chemotherapy. In fact, of their seven responders, only two had had previous chemotherapy. Cruz et al reported seven responses of 25 evaluable ovarian adenocarcinomas treated with 100–130 mg/m² of CCNU every 6 weeks, but it is not clear what the prior drug treatment may have been. Neither report gives any suggestion of a better therapeutic index than mustards. In contrast, Jorgensen et al (7) employed another nitrosourea, BCNU, which appears to have a similar spectrum and level of anticancer activity (1,2). They randomized patients to receive cyclophosphamide or BCNU. Ten of 11 patients with ovarian adenocarcinoma progressed with BCNU while only five of 11 patients treated with cyclophosphamide showed disease progression during the trial.

It is possible that the doses of CCNU and methyl-CCNU in our trial could have been higher but, on the one hand, the dose of CCNU is comparable to that previously used (5,6) and, on the other hand, our patients had had extensive prior treatment. It is unlikely that higher doses would have been tolerated by many patients. If all our evaluable patients treated with CCNU were pooled with those of Cruz and Sasaki, there was an 18% complete plus partial response rate (seven of 18, plus seven of 25, plus none of 31 or 14 of 74). As noted above, this modest response rate represents activity primarily in patients without prior drug therapy. We found no advantage with methyl-CCNU. If the nitrosoureas have a role in treating ovarian adenocarcinoma, it might be in combination with other active agents, but the use of these drugs as single agents in mustard failures is unproductive.

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Whole-Body Computed Tomography in the Diagnosis of Abdominal and Thoracic Malignancy: Review of 600 Cases [1]

Everett G. Schaner,* Gordon L. Head, Matthew A. Kalman, N. Reed Dunnick, and John L. Doppman [2,3]

SUMMARY

Whole-body computed tomography (CT) was performed on 600 patients with known or suspected malignancy at the National Institutes of Health. This new diagnostic modality utilizes a finely columnated X-ray source mounted opposite a computer-linked detector bank to generate anatomic cross-sectional images of the body part being scanned. CT proved valuable in defining many pathologic processes and was, at times, deemed the only test short of exploratory surgery that yielded diagnostic information. This latter benefit was most acutely emphasized in lesions affecting the retroperitoneum, adrenal glands, pelvis, liver, and immediate subpleural pulmonary parenchyma.

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The impact of computer-assisted cranial tomography on the fields of radiology, neurology, and neurosurgery has been dramatic (1–3). The unique and noninvasive visualization of normal intracranial anatomy and pathologic alterations that tomography affords is unequaled by any previous diagnostic modality and accounts for its ready acceptance. Approximately 3 years ago the production of prototype whole-body computed tomography (CT) scanning units heralded the use of this technology for the detection of intra-abdominal and intrathoracic disease since it provides an accurate reconstruction of the internal structures of the body. Indeed, several preliminary reports have already appeared emphasizing the value of whole-body CT in the diagnosis, staging, and management of intra-abdominal and intrathoracic malignancy (4–9).

Since May of 1976, an EMI CT-5000 prototype body scanner has been in operation in the Department of Radiology, CC, NIH. To date, > 600 examinations have been performed, the majority being in patients with known or suspected intra-abdominal or intrathoracic malignancy. This report details our cumulative experience with particular emphasis being given to the use of whole-body CT in detecting and explicitly defining both primary and/or metastatic disease.

Because our case material was principally drawn from patients with documented pathology, it is not our intention to statistically compare the efficacy of CT versus other diagnostic modalities vis-à-vis establishing any given diagnosis. Rather, we wish to illustrate the range of pathology which can be visualized by CT and, where applicable, underscore those diagnostic benefits that CT can yield.

MATERIALS AND METHODS

The EMI CT-5000 whole-body scanner consists of a scanning gantry supporting a fixed anode, oil-cooled X-ray tube and a bank of 30 NaI crystal detectors. The X-ray source and detector heads maintain a fixed relationship opposite each other as they pass across the patient (fig 1A). After each traverse of the beam-detector bank, the scanning frame indexes 10° and the traversing process is repeated. Traverses are performed 18 separate

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[2]Diagnostic Radiology Department, Clinical Center (CC), National Institutes of Health (NIH), Bethesda, Md.

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*Reprint requests to: Everett G. Schaner, MD, Diagnostic Radiology Department, CC, Bldg 10, Rm 6S211, NIH, Bethesda, Md 20014.

times through an arc of 180° (fig 1B). The scanning process takes 18 seconds.

Approximately 324,000 separate X-ray absorption coefficient values are logged by the detector bank during a 180° scanning sequence. These values are then subjected to an analog to digital conversion and are processed by computer to generate a 320 × 320 matrix of X-ray absorption coefficient values. Each matrix element (referred to as a pixel) corresponds to a volume of tissue 1 × 1 × 13 mm in dimension for the 13-inch scanning field that is routinely employed in body imaging. The matrix is displayed on a cathode ray tube as shades of gray ranging from black (areas of low absorption) to white (areas of high absorption). The resulting image can be manipulated by the viewer to magnify regions of interest and to determine their average absorption coefficient values. A complete study usually comprises from eight to 12 transverse scans with each "slice" representing a volume of tissue 13 mm thick. Scans are stored for later recall on magnetic tape and permanent polaroid images of any individual "slice" can be obtained with a Shakman camera fixed to an additional cathode ray tube monitor.

This imaging system will theoretically allow definition of lesions 1 mm in dimension using a 13-inch scanning field and a 320 × 320 matrix image display, assuming that the absorption coefficient of the lesion is significantly different from that of the immediately surrounding tissues. Such differences can sometimes be enhanced by the iv infusion of iodine-containing contrast material whose high atomic number (relative to hydrogen, oxygen, and carbon) produces a definite increase in X-ray attenuation. As an example, the spatial resolution of a small avascular metastasis in the vascular hepatic parenchyma will be increased as the liver tissue concentration of contrast medium increases after infusion. For this reason, most of our patients have been scanned following the iv infusion of 100 cc of renografin 60. An absorbed radiation dose of approximately 4 rads[4] is delivered during an average examination using the EMI CT-5000 scanner. This is certainly deemed within acceptable levels, especially given the precise anatomic data it provides. By comparison, a barium enema with fluoroscopy results in an absorbed dose of 15–20 rads.

In the course of our early trials with CT body scanning we have principally studied hospitalized patients receiving therapy protocols being conducted by both the medical and surgical arms of the NCI. They represent a broad spectrum of malignancy and the results of their scans form the basis for this report.

RESULTS

Table 1 provides a statistical breakdown of CT scans performed in > 600 patients with known or suspected thoracic or abdominal malignancy.

Retroperitoneum

The retroperitoneum with its vascular, nodal, fatty, and muscular components is a difficult area to evaluate with conventional radiography. Lymphography, arteriography, and venography are the modalities usually employed to identify retroperitoneal lesions. These procedures are, however, uncomfortable for the patient and in some instances carry attendant risks. Conversely, the retroperitoneum is readily amenable to examination by CT since the nodal, vascular, and muscular components are directly visualized by virtue of the lower absorption coefficient of the surrounding, and therefore demarcating, layers of retroperitoneal fat.

Retroperitoneal Lymph Nodes in Lymphoma

Positive CT in 35 patients with abdominal lymphoma has demonstrated its superior ability vis-à-vis bipedal lymphography in outlining the total

TABLE 1.—Statistical breakdown by organ scanned and/or clinical diagnoses in approximately 600 cases utilizing the EMI CT-5000 whole-body scanner

Organ	No. of patients
Liver	133
Retroperitoneum	
Lymphoma	63
Other sarcomas	24
Gonadal carcinoma	13
Burkitt's lymphoma	11
	111
Chest	62
Pancreas	58
Mediastinum	53
Kidneys	52
Pelvis	
Ovarian carcinoma	27
Other pelvic masses	22
Spine, bone, extremities	41
Adrenal glands	26
Neck	19
Total	604

[4]Kirkland LR. Radiation Oncology Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute (NCI). Personal communication.

volume of para-aortic nodal mass (fig 2), as well as defining the complete cephalad extent (fig 3) of the lymphomatous process. In addition, associated lymphomatous extension into renal and splenic hilar nodes or the mesenteric root (fig 4) is defined by CT as is any associated splenomegaly (11 patients) or focal splenic lymphomatous deposit (four additional cases) (10). Further, Kreel (11) has reported two instances where lymphomatous masses were defined in the superior reaches of the retroperitoneum in patients whose immediately preceding lymphangiograms were normal. CT, therefore, proves valuable in both tumor staging and radiotherapy planning (12,13). Sequential scans have been successfully utilized to quantitate response in patients undergoing therapy where the presence of bowel gas precludes technically acceptable ultrasound examinations.

Conversely, our experience thus far suggests that CT, at its present level of sophistication, is unable to distinguish between normal and lymphomatous nodes where nodal enlargement is absent. Therefore, although CT will not replace lymphography as the initial diagnostic modality in defining abnormal nodal architecture, it has proved more sensitive than lymphography in determining the true extent of disease when gross changes are present.

Retroperitoneal Sarcomas

Twenty-four patients with retroperitoneal sarcoma were examined. Nine had positive scans. This group included patients with liposarcoma, rhabdomyosarcoma, and undifferentiated small cell sarcoma. Direct invasion of the retroperitoneal musculature and liver was seen in one patient with small cell sarcoma and extension of tumor into contiguous vertebral bodies with accompanying compression of the adjacent spinal canal (fig 5) was documented in two cases. Retroperitoneal liposarcomas were easily defined by virtue of their low absorption coefficients (–5 to –20 EMI) in comparison to surrounding normal tissues. This, of course, resulted from the high fat content of these tumors (fig 6).

Retroperitoneal Lymph Nodes in Gonadal Carcinomas

Thirteen patients with testicular carcinoma were examined; 11 exhibited retroperitoneal and/or intra-abdominal pathology. CT proved valuable in staging these patients by documenting the extent and location of their metastatic disease. This aided surgical planning prior to debulking procedures. As with lymphomatous involvement of the retroperitoneal nodes, bipedal lymphography underestimated the volume and extent of nodal invasion actually taking place (10).

The tendency for metastatic testicular carcinoma to cavitate centrally, an observation often made at surgery, was an interesting phenomenon demonstrated in six of the 13 patients scanned (fig 7).

CT examinations of the abdomen were performed in 15 patients with ovarian carcinoma following bipedal lymphography to determine whether CT could replace lymphography as the primary screening modality in detecting nodal metastatic involvement. Although in a single instance a non-opacified tumor-infiltrated nodal mass was visualized in the mid-lumbar area above the level of opacified nodes, it is our general feeling that CT is not particularly useful in detecting early nodal pathology in this disease since ovarian metastases to lymph nodes are not usually accompanied by nodal enlargement.

Pelvis

A comparison of CT and ultrasound has defined complementary roles for each diagnostic modality in denoting the location and nature of pelvic masses. Although the ultrasound study can provide a somewhat better differentiation of solid from cystic lesions, the CT scan provides precise anatomy of both the soft tissue and skeletal components of the pelvis (14).

CT examination of the pelvis was performed in 27 patients with ovarian carcinoma. Two of these were performed in the course of the initial staging workup with the remainder being undertaken as part of the individual patient's continuing followup for recurrent disease. Fifteen patients had pelvic masses confirmed by ultrasound. The ovarian tumors visualized were usually large and either supravesical or retrovesical in location. Eight of the 15 carcinomas were cystic or multicystic in appearance (fig 8). Tumor recurrence deep within the "true" pelvis in patients who were post radical hysterectomy was easily detected, and the relationship of disease to the recto-sigmoid colon and distal ureters was defined (fig 9). CT examination superseded gray scale ultrasound as the procedure of choice in following tumor response to therapy in several patients in whom chemotherapy-induced bowel ileus yielded technically inadequate ultrasound scans. Additionally, clinically unsuspected and previously undiagnosed extraperitoneal (anterior abdominal wall) and retroperitoneal tumor implants were defined in two patients undergoing CT examinations of the pelvis for the first time (fig 10).

Eleven patients with Burkitt's lymphoma were examined; six of these were studied as part of their initial staging evaluation. Pelvic masses were present in three patients, and central tumor necrosis was noted in one of these (fig 11). In each case,

radiographic evidence of mass was also present. However, the dimensions and extent of the pathology had been significantly underestimated.

Twenty-two additional patients underwent CT examinations of the pelvis for varying reasons. Seven patients had normal CT scans. A pelvic mass was found in the remaining 15 patients. Of these 15, five patients had an underlying and previously detected primary malignancy elsewhere within the pelvis and the lesions uncovered were therefore interpreted as probable metastatic foci. Pelvic multicystic masses with appearances typical of ovarian carcinoma were detected in two additional patients with other previously diagnosed abdominal malignancies. The presence of a second primary was therefore suggested and subsequently confirmed surgically. Solid pelvic masses were noted in six patients with no known underlying malignancy. Three of these patients were subsequently explored and the masses represented a pre-sacral neurolemma with central necrosis and dystrophic calcification (fig 12), a hemorrhagic ovarian cyst, and a pre-sacral focus of extramedullary hematopoiesis. Histologic diagnoses are not available for the remaining three patients. An unexpected pregnancy was diagnosed in one patient, a 21-year-old woman with fibrosarcoma being evaluated for suspected metastatic disease to the ischial tuberosity and adjacent soft tissues. Finally, a large right-lower quadrant ovarian cyst (surgically proven) was incidentally diagnosed in a young woman with lymphoma who was being studied to assess the extent of her pelvic adenopathy (fig 13).

Adrenal Glands

The normal left adrenal gland is visible in approximately 80% of patients undergoing CT scanning (fig 14). The right adrenal gland is seen less frequently (50%). Visualization is dependent upon the presence of retroperitoneal fat whose lower absorption coefficient silhouettes the structure. As the quantity of investing fat increases, the likelihood of visualization is enhanced. Adrenal masses including adenomas, adrenal carcinomas, pheochromocytomas, aldosteronomas, and metastases to the adrenal glands are readily visualized even when their size is in the 1–2-cm range, because of their propensity to distort the gland's anatomy. Twenty-five of our patients were scanned to specifically evaluate the adrenal glands for pathology. Additionally, an unsuspected large adrenal mass was incidentally demonstrated in a patient undergoing an abdominal scan for other reasons.

CT scans in three patients with Cushing's syndrome revealed left adrenal adenomas. In one of these, massive obesity had precluded angiographic evaluation and tomography had been equivocal. CT, therefore, proved to be the definitive diagnostic modality (fig 15). In two additional patients with clinical hyperaldosteronism, CT outlined a left adrenal mass lying anterior and medial to the upper pole of the kidney (fig 16). These lesions were not visualized on the iv pyelogram which was supplemented with tomography. Their presence was subsequently confirmed by adrenal venography.

Five patients with symptoms of pheochromocytoma were studied and two had positive scans. In one patient, a left pheochromocytoma had been resected 3 years previously. CT revealed a recurrent tumor lying medial and anterior to the left renal hilum. The absorption coefficient value of this lesion markedly increased following contrast infusion, indicating its vascular nature (fig 17) subsequently confirmed at arteriography. In the same patient, a hypervascular metastatic focus was additionally seen in the left posterior mediastinum (fig 18). One false-negative scan was obtained when a pheochromocytoma of the right adrenal gland could not be outlined due to a lack of retroperitoneal fat. This lesion was, however, easily defined by ultrasound.

In four patients with pre-existing adrenal carcinoma CT demonstrated recurrent retroperitoneal disease. The extent of tumor recurrence was accurately gauged by CT. In one of these patients a portion of the tumor had previously been therapeutically embolized, and the margin between viable and necrotic tumor was evident after contrast infusion (fig 19). By performing sequential scans in these patients, information regarding the rate of regrowth of residual tumor was obtained.

One patient with congenital adrenal hyperplasia and three patients who were examined because of suspicious findings on their excretory urograms had negative scans precluding further evaluation. Finally, clinically unsuspected metastatic disease in the left adrenal gland was incidentally discovered in a patient with malignant melanoma (fig 20).

Kidney

The kidneys are well defined at CT by virtue of their investment with retroperitoneal fat. Significant additional information regarding the renal parenchyma and pelvo-calyceal systems can be obtained by performing the scans after the iv infusion of contrast material. Although most renal pathology is apparent with high-quality excretory urography, CT has proved a useful tool in distinguishing solid from cystic masses, particularly when multiple small lesions are present (fig 21). Simple benign

cysts are of homogeneous absorption (+5 to +12 EMI) and interface sharply with surrounding renal parenchyma. However, renal carcinoma distorts the renal capsule, invades the retroperitoneum, and interfaces poorly with neighboring functioning renal tissue.

To date, 16 scans have been performed specifically to exclude or confirm the presence of a suspected renal mass. Nine of these were negative, precluding any further evaluation. Five patients had solid mass lesions. In one of these, which subsequently proved to be a highly vascular hypernephroma, CT defined both retroperitoneal invasion (fig 22) and a large renal vein which we believed reflected large-volume arteriovenous shunting within the lesion. In the remaining two patients, multiple simple cysts and sinus lipomatosis were observed.

Sixteen simple renal cysts were incidentally discovered in the course of scanning for other intra-abdominal pathology. Most of these were so classic in their appearance with absorption coefficient values in the +5 to +12 EMI unit range that we felt no additional evaluation was warranted.

CT has proved useful in identifying the extent of subcapsular and perirenal bleeding (15). Three patients were examined who developed symptoms suggestive of retroperitoneal bleeding following percutaneous renal biopsy. Two perirenal (fig 23A) and one subcapsular (fig 24A) hematoma were defined. Followup examinations revealed a decrease in both the size and absorption coefficient of each collection confirming its progress towards resolution (figs 23B and 24B). CT provided information regarding the hematoma's inferior extent, volume, and relationship to functioning parenchyma. To confirm the sensitivity of CT scanning in detecting early retroperitoneal bleeding, three asymptomatic patients were scanned following percutaneous renal biopsy. In each, a small perirenal hematoma was noted adjacent to the site of biopsy (fig 25), suggesting that CT may represent the earliest indicator of developing retroperitoneal hemorrhage. Finally, hydronephrosis was noted as an incidental finding in 12 patients being scanned to determine the extent of known retroperitoneal lymphoma or testicular carcinoma. In several patients, direct tumor extension into the renal hilum was documented (fig 26) although it had not been suspected on either urography or tomography. This information was valuable in providing proper radiotherapy planning.

Liver

Normal livers are homogeneous in density although one lobe may vary greatly from the others in both size and topographic anatomy. Lesions within the liver are usually less dense than the surrounding normal hepatic parenchyma. This difference can be accentuated by the iv infusion of iodine-containing contrast material. Liver cysts (fig 27), solitary and multiple metastases, and primary hepatic neoplasms have all been well demonstrated by CT (16). CT is particularly valuable in identifying small lesions (down to 5 mm in diameter) deep within the hepatic substance and therefore not usually visible with nuclear scanning (fig 28). However, a specific limitation in the detection of intrahepatic lesions by CT occurs with hypervascular masses occupying a significant portion of the liver substance. This principally includes large hepatomas and hypervascular metastases. Such lesions tend to interface poorly with the surrounding normal hepatic parenchyma and, following contrast infusion, any initial difference in the absorption coefficients between tumor and normal tissue may be secondarily masked. This suggests the necessity of performing pre- and post-contrast scans in patients suspected of harboring such lesions.

Since patients demonstrating either clinical or laboratory evidence of hepatic metastases are generally not admitted to the NIH for protocol evaluation, most of our patients who received liver scans (128 patients) have yielded normal studies. Hepatic metastases have been defined in two patients with islet cell carcinoma of the pancreas, one patient with colorectal carcinoma (fig 29), and another patient with melanosarcoma.

Two patients with hepatoma have been evaluated; in each, the lesion was large, poorly defined, and blended with the surrounding hepatic parenchyma following contrast infusion (fig 30A and B).

CT has been found to be of specific value in distinguishing medical from surgical jaundice. It provides a rapid and noninvasive means of visualizing dilated intrahepatic ducts and their radicles (fig 31A and B) and can be used in critically ill patients with jaundice in whom more invasive procedures such as transhepatic cholangiography cannot be performed.

Enlargement of the gallbladder and mild intrahepatic duct dilatation secondary to periportal adenopathy were noted as incidental findings in three patients with lymphoma and in one patient with melanoma (fig 32). These observations were made prior to the development of clinical jaundice and dictated a vigorous and specific course of therapy.

In addition to metastatic disease, abnormal liver storage of iron can be identified and quantitated. Twenty-seven patients with hemochromatosis have been examined and the increased absorption coeffi-

cient of stored iron within the hepatic parenchyma has been correlated with liver biopsy specimens (fig 33) (17). An extension of this approach to the evaluation of iron deposition within the myocardium and the response of abnormal iron stores to therapy is now under investigation.

Pancreas

The normal pancreas cannot be visualized using any conventional radiographic technique short of angiography. Solid mass lesions, pseudocysts, and diffuse pancreatic enlargement will produce compression and distortion of the adjacent gastric antrum and duodenal sweep and can be documented with the appropriate barium examination. However, until the advent of gray scale ultrasonography, the nature of the pathologic process could not be directly identified. The normal pancreas is visualized utilizing CT in patients with a normal quantity of retroperitoneal fat (fig 34). As the quantity of retroperitoneal fat increases, gray scale ultrasound examination becomes more difficult to perform, whereas visualization of the gland by CT is facilitated. Conversely, in the thin or emaciated patient, gray scale ultrasound affords excellent visualization of the organ while its margins cannot be identified by CT.

Initial enthusiasm referrable to the early detection of carcinoma of the pancreas utilizing CT has been somewhat dampened since the realization that the absorption coefficient value of tumor tissue does not vary from that of normal surrounding pancreas. To allow detection, the lesion must be large enough to distort the anatomy of the gland, invade the neighboring retroperitoneal fat, or produce ductal obstruction (18). Hypervascular lesions including B- and non-B-islet cell tumors are, at times, identifiable following contrast infusion even if their bulk does not distort the margins of the gland. They do, however, represent a minority of pancreatic lesions.

Twenty-two patients with proven carcinoma of the pancreas have been examined. All of these patients had prior exploratory surgery and a significant number had bypass procedures for relief of biliary obstruction. Of the 22 patients scanned, 14 had definite or probable CT evidence of a pancreatic mass identifiable by virtue of the lesion's bulk (fig 35). Tissue planes about the pancreatic head were often difficult to identify secondary to tumor invasion and previous surgery. Lesions in the body (fig 36) and tail (fig 37) of the pancreas were easier to localize in relation to neighboring vascular and retroperitoneal structures than those lesions confined to the head.

Twenty-one patients were scanned specifically for evaluation of islet cell tumor. CT scanning demonstrated pancreatic pathology in eight patients. In four of these eight patients the lesions enhanced following contrast infusion (fig 38). In addition to solid tumors of the pancreas, pseudocysts were defined both in association with chronic calcific pancreatitis (fig 28) and carcinoma of the pancreas. In a single patient, a dilated central pancreatic duct was identified secondary to an obstructing mass in the pancreatic head.

Since our experience with CT of the pancreas has been confined primarily to patients with surgically documented lesions, its use has been primarily directed towards defining tumor size and extrapancreatic extension. One practical consideration of importance has been the consistent visualization of the lesions in question despite ileus in adjacent bowel loops which results from the chemotherapy these patients receive and which hampers adequate ultrasound evaluation.

Chest and Mediastinum

The bony thorax and the structures it contains can be accurately evaluated by conventional radiography, particularly when accompanied by high-quality tomography. CT has, however, found specific application in the study of chest wall lesions and in the detection of small peripheral pulmonary nodules (19). Since the absorption coefficient of chest wall masses differs significantly from that of the surrounding subcutaneous fat or aerated lung, these lesions are readily visible. Chest wall and pleural masses have been examined in nine patients. In addition to visualizing the lesion under investigation (fig 39), CT has documented accompanying pericardial extension, sternal destruction, and central tumor necrosis. CT was superior to anteroposterior and lateral tomography in mapping tumor margins for radiotherapy in each of these nine lesions.

Fifteen young patients with appendicular soft tissue or osseous sarcomas and melanoma have received full chest (lung) CT examinations in an effort to define additional peripheral nodules not noted on conventional whole-lung anteroposterior tomography. Such nodules, primarily pleural and subpleural in location and ranging in diameter from 3 to 6 mm, were found in most patients yielding roughly a twofold increase in sensitivity of CT over conventional tomography in defining parenchymal nodules (fig 40A, B, and C). At thoracotomy, several of these nodules were indeed small metastases. However, most (approximately 65%) in the 3–6-mm range proved to be benign nodes and granulomas

(20), a finding which underscores a specific limitation of CT at its present level of sophistication, ie, lack of tissue specificity.

Numerous small subpleural metastases, not surgically confirmed, were also found in four patients with testicular carcinoma. CT defined bilateral metastatic disease in the posterior gutters not visible, even in retrospective analysis, on conventional tomography (fig 41A and B), a contribution of obvious importance in staging and treatment planning.

Fifty patients with parenchymal pathology including advanced idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, Wegener's cavitating pulmonary nodules, and primary pulmonary malignancies such as small cell anaplastic and squamous bronchogenic carcinomas were evaluated to assess the contribution CT could offer in diagnosis and management. CT provided no discernible benefit beyond conventional radiography and tomography in such cases.

Fifty-three patients underwent CT examination of the mediastinum to exclude or identify either a thymoma or a parathyroid adenoma. Those in whom an adenoma was clinically suspected were hospitalized because of well-established but unexplained hyperparathyroidism. Those patients in whom thymomas were to be excluded were individuals suffering from either myasthenia gravis or congenital immunodeficiency. In both groups, routine posteroanterior and lateral chest roentgenograms were negative with regard to the presence of a mediastinal mass. Four anterior mediastinal parathyroid adenomas were found (fig 42), and subsequently confirmed at angiography (21).

Bone

Skeletal System and Extremities

CT affords excellent visualization of the skeletal system. It has found specific application in evaluation of the spinal canal, vertebral column, bony pelvis, and long bones of the extremities.

Thirty-two patients were evaluated specifically to confirm or exclude bony lesions in the vertebral column and pelvis. Eleven patients had positive CT scans. Blastic metastases from lymphoma (fig 5) and carcinoid and lytic metastases from melanoma, lymphoma, malignant islet cell tumor, and oat cell carcinoma of the lung (fig 43) were identified. Invasion of the epidural space and partial compression of the spinal canal by metastatic carcinoma and lymphoma have also been documented (fig 5).

The intrapelvic soft tissue components of Ewing's sarcoma and chondrosarcoma (fig 44) originating in the iliac wings were visualized in six patients in whom followup CT scans during chemotherapy documented regression of the intrapelvic soft tissue tumor mass.

CT is also finding specific application in the diagnosis of spinal stenosis and spinal cord and nerve root compression by a variety of benign and malignant processes. Visualization of the spinal cord and nerve roots has been further facilitated by the recent introduction of a new water-soluble iodine-containing myelographic contrast medium, metrizamide (22).

Osseous tumors of the extremities are now coming under investigation by CT. Although conventional radiographs are usually diagnostic, CT appears useful in defining the extent of soft tissue and intramedullary invasion by such malignancies of the bone. Extremity soft tissue sarcomas are also readily defined since they distort and invade surrounding muscle groups, fascial planes, and neighboring subcutaneous fat (fig 45).

DISCUSSION

In reviewing the CT scans of > 600 patients, most with documented intrathoracic and/or intraabdominal pathology, our experience to date suggests that CT plays both a primary and complementary role in the diagnosis and management of patients with known or suspected malignancy. Since it allows direct visualization of normal anatomic structures as well as pathologic processes, it provides, in conjunction with gray scale ultrasound, knowledge of the texture and internal architecture of lesions under investigation. It, therefore, readily distinguishes cystic from solid masses and defines the presence of compression and/or invasion of surrounding structures. Further, with the aid of injected contrast, solid lesions can be further categorized into hypovascular or hypervascular masses, all findings which will bear upon the correct diagnosis.

CT is more accurate than lymphangiography in defining retroperitoneal pathology, especially diffuse and extensive lymph node invasion in patients with lymphoma and metastatic testicular carcinoma. It is a rapid and accurate method for distinguishing medical from surgical jaundice and it enables the identification of deep-seated intrahepatic metastases. Although our experience with CT in the evaluation of pancreatic neoplasms has been confined primarily to surgically proven lesions, it has proved valuable in documenting tumor growth during therapy, particularly periportal and retropancreatic extension.

Although both renal and adrenal pathology can

be documented at angiography, this procedure carries specific morbidity. However, with noninvasive CT we have been able to define adrenal lesions, both benign and malignant, the location and extent of which had not been suspected utilizing conventional tomography. CT superseded gray scale ultrasound in identifying such lesions, particularly in obese patients and in individuals with an abundance of bowel gas. Additionally, the retroperitoneal extension of renal carcinoma, the posterior and inferior extent of perirenal retroperitoneal bleeding, and the presence of multiple small intrarenal cysts were all visualized with an accuracy deemed by us to preclude further diagnostic procedures.

CT proved a useful adjunct in evaluating tumors of ovarian and uterine origin. This was most apparent in its ability to locate lesions deep within the cul-de-sac and accurately predict their internal architecture. Furthermore, the intrapelvic soft tissue component of sarcomas of the bony pelvis can be readily mapped for radiotherapy and subsequently followed towards resolution. Finally, prospective studies now underway suggest a limited but specific application for CT in defining small pleural and subpleural pulmonary metastatic nodules and pleural and chest wall extension of neoplasms in selected individuals.

In conclusion, CT has added a new and as yet not fully explored dimension to the evaluation of thoracic and abdominal pathology. As our experience increases with this new imaging tool, its ability to provide anatomic cross sections should secure for it a place of increasing prominence in radiologic diagnosis.

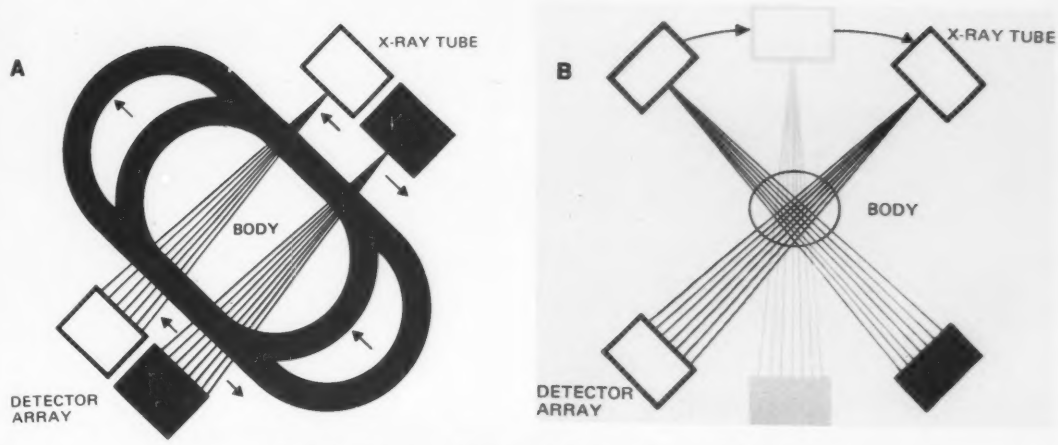

FIGURE 1.—Schematic drawing of the beam-detector bank traverse (A) and index (B) principle utilized in the EMI body scanner.

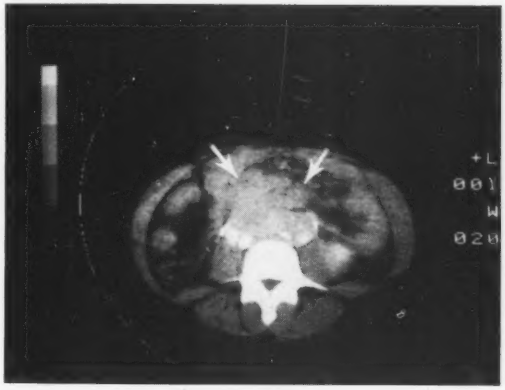

FIGURE 2.—33-yr-old woman with histiocytic lymphoma. Enlarged opacified prevertebral nodes ring the posterior margin of a large unopacified prevertebral nodal mass (→).

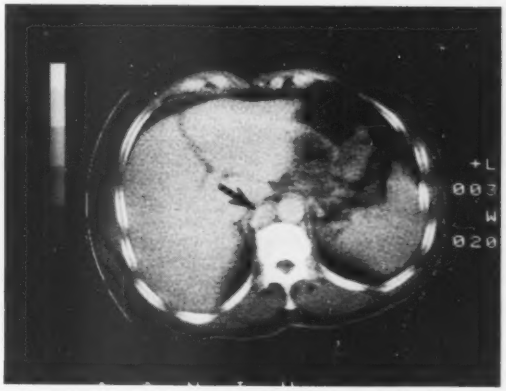

FIGURE 3.—46-yr-old man with abdominal lymphoma. Enlarged lymphomatous node (→) bows the right crus of the diaphragm indicating tumor extension into the posterior mediastinum.

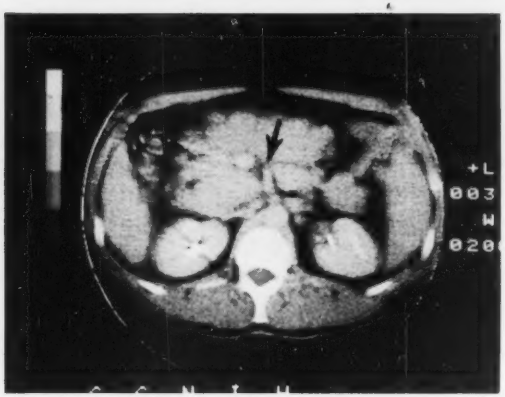

FIGURE 4.—41-yr-old man with nodular lymphoma. Lymphomatous masses are noted extending into both renal hila and into the mesenteric root (→).

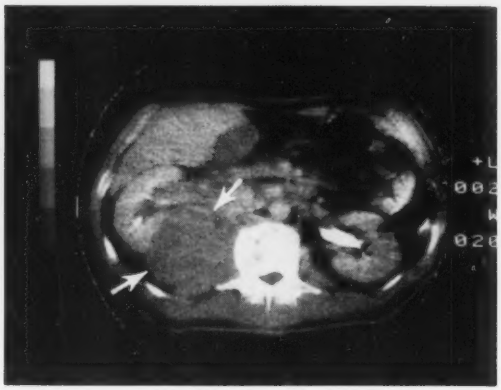

FIGURE 5.—51-yr-old man with small cell sarcoma. A right retroperitoneal mass is noted (→) pushing the right kidney anteriorly and laterally, invading the right psoas muscle and L2 vertebral body, and producing partial compression of the spinal canal at this level.

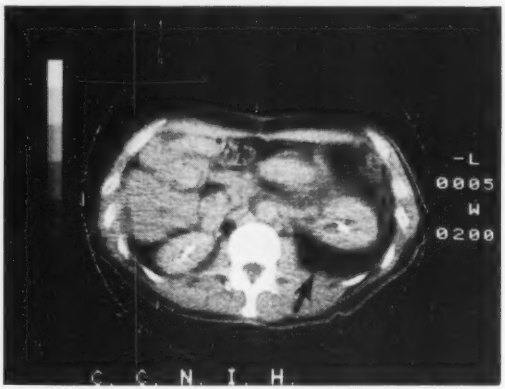

FIGURE 6.—46-yr-old man with a left retroperitoneal liposarcoma. Note anterior displacement of left kidney by a crescentic mass of low absorption coefficient (→) indicating high fat content.

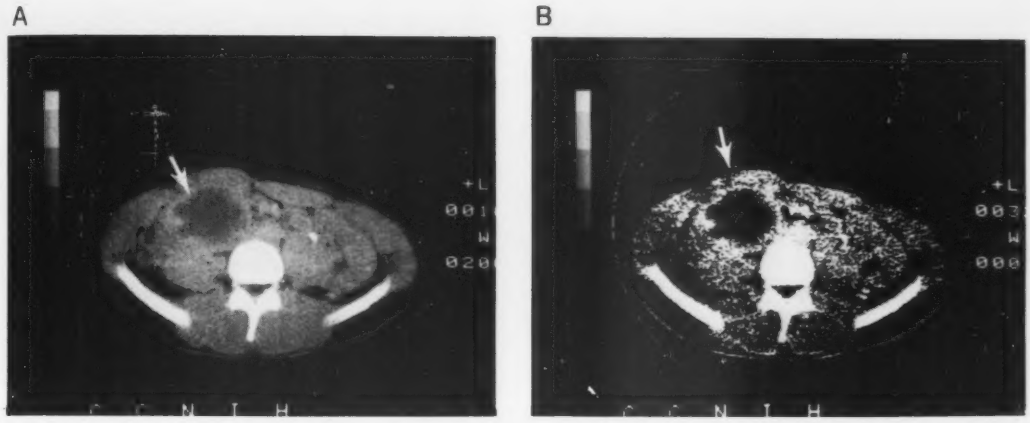

FIGURE 7.—26-yr-old man with testicular carcinoma. A large right retroperitoneal mass is evident with central cavitation (→). (A) Standard mode. (B) "Measure" mode utilized to determine X-ray adsorption coefficient (μ) values of the lesion.

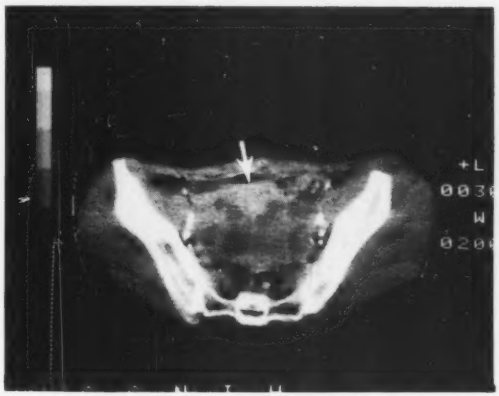

FIGURE 8.—53-yr-old woman with ovarian carcinoma. A multicystic mass is noted (→) filling the pelvis.

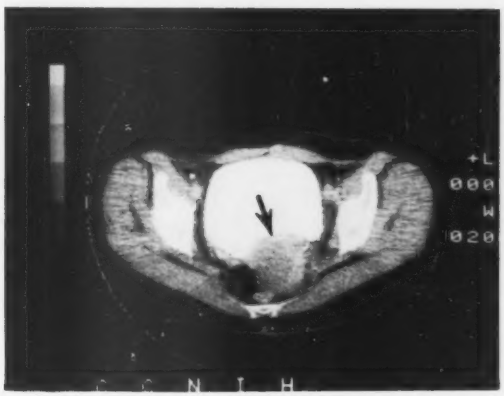

FIGURE 9.—31-yr-old woman with ovarian carcinoma status post radical hysterectomy. Tumor recurrence is present deep within the cul-de-sac (→) indenting the posterior margin of the bladder and displacing the rectum towards the right.

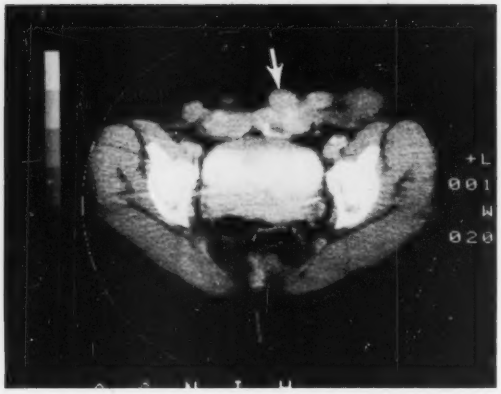

FIGURE 10.—34-yr-old woman with ovarian carcinoma. In addition to a pelvic mass, subcutaneous tumor implants are evident along the anterior abdominal wall (→).

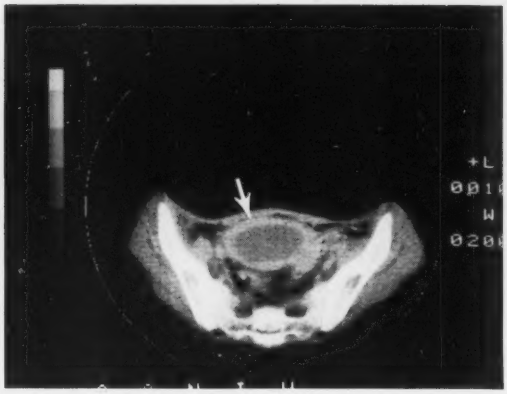

FIGURE 11.—12-yr-old girl with Burkitt's lymphoma. A supravesical mass is present with central cavitation (→).

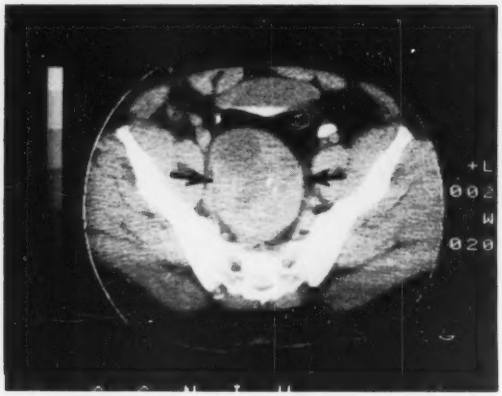

FIGURE 12.—42-yr-old man with clinically suspected pelvic mass. A well-defined ovoid mass with central necrosis and calcification is noted (→) contiguous with the sacrum. This proved to be a pre-sacral neurolemma at surgery.

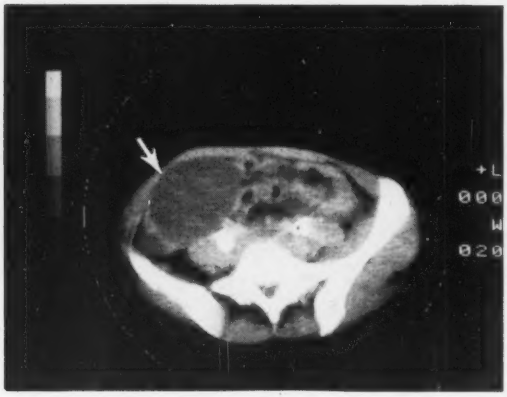

FIGURE 13.—33-yr-old woman with lymphoma. In addition to enlarged lymphomatous nodes in the pelvis, an unsuspected large right ovarian cyst is evident (→).

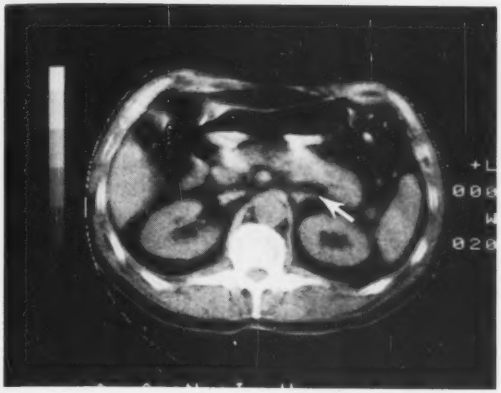

FIGURE 14.—Transverse CT scan of the upper abdomen demonstrating a normal left adrenal gland (→).

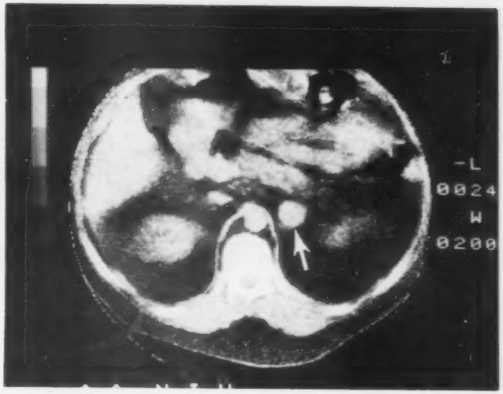

FIGURE 15.—46-yr-old man with Cushing's syndrome. A left adrenal adenoma is present (→) surrounded by an extensive bed of retroperitoneal fat.

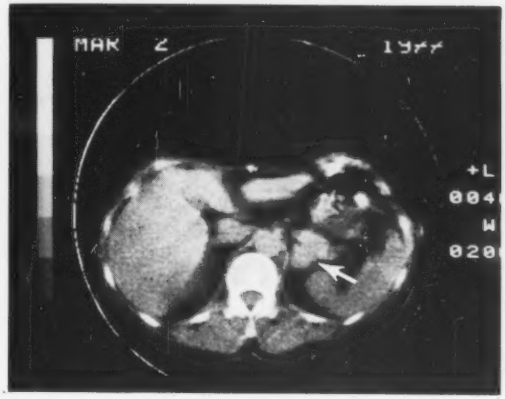

FIGURE 16.—46-yr-old woman with symptoms of hyperaldosteronism. An aldosteronoma of the left adrenal gland is noted lying anterior to upper pole of the left kidney (→) and therefore not distorting its axis on conventional tomograms.

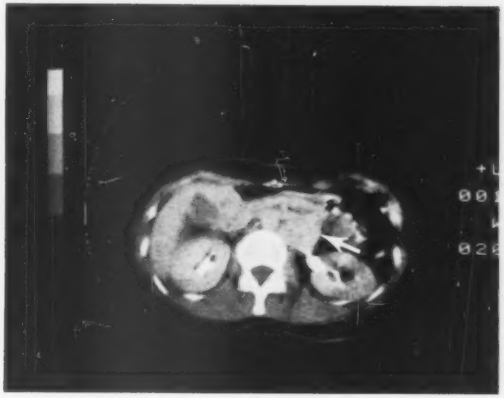

FIGURE 17.—38-yr-old woman with symptoms of recurrent pheochromocytoma. A mass (→) which enhanced following contrast infusion is present medial to the left renal hilum. It proved to be recurrent malignant pheochromocytoma.

FIGURE 18.—Same patient as noted in fig 17. A left paramediastinal mass is defined (→) which enhanced dramatically after contrast infusion and proved to be metastatic pheochromocytoma.

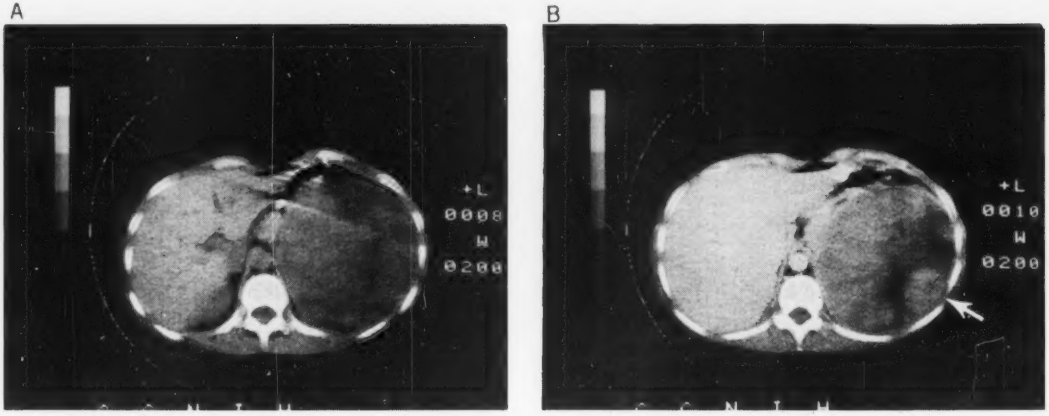

FIGURE 19.—(A) Pre- and (B) post-contrast CT scans in a 21-yr-old woman with a large left upper quadrant adrenal carcinoma status post-arterial embolization. Following contrast infusion, still viable nodules of vascularized tumor are evident (→). However, tumor necrosis is seen in the distribution of the previously embolized vessels.

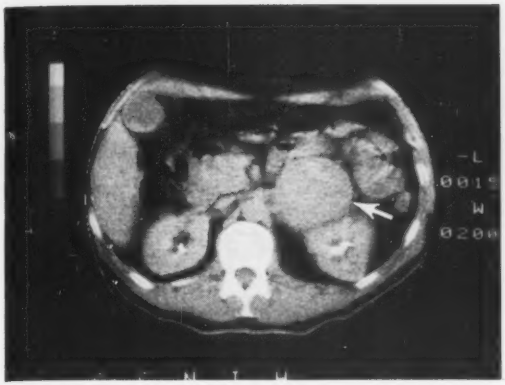

FIGURE 20.—24-yr-old man with metastatic melanoma. A large unsuspected metastasis in left adrenal gland is noted (→).

FIGURE 21.—44-yr-old man with bilateral intrarenal masses. CT scan confirms the presence of small bilateral intrarenal cysts (→).

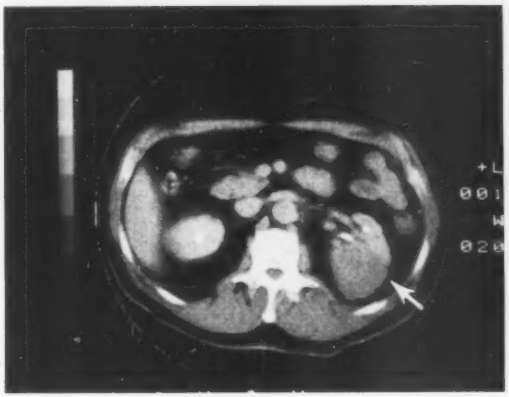

FIGURE 22.—53-yr-old man with suspected left renal mass. A solid mass is present (→) distorting the left renal contour and invading the left retroperitoneum; it proved to be renal carcinoma.

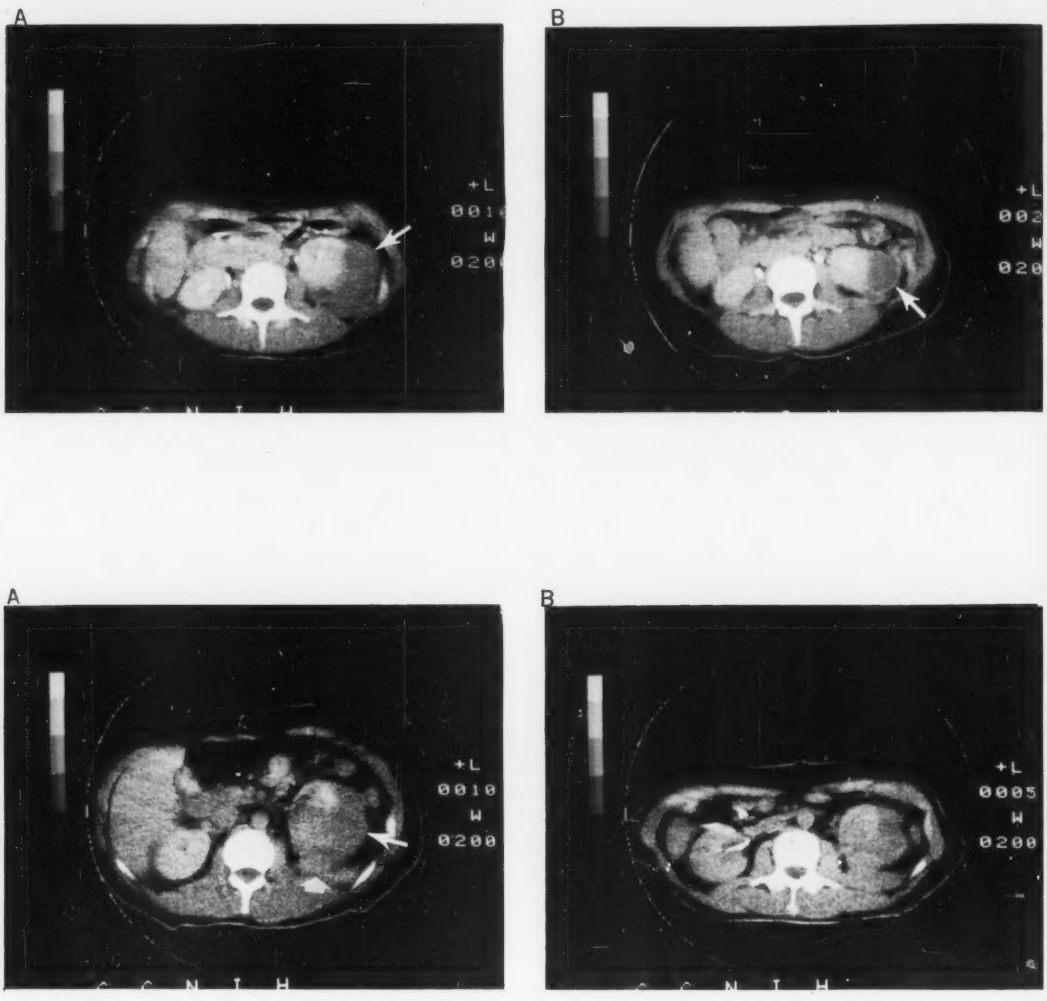

FIGURE 24.—47-yr-old man with symptoms of retroperitoneal bleeding following percutaneous renal biopsy. (A) CT scan defines both subcapsular (→) and posterior perirenal hematoma (◊). (B) 7 days later both collections are undergoing resolution.

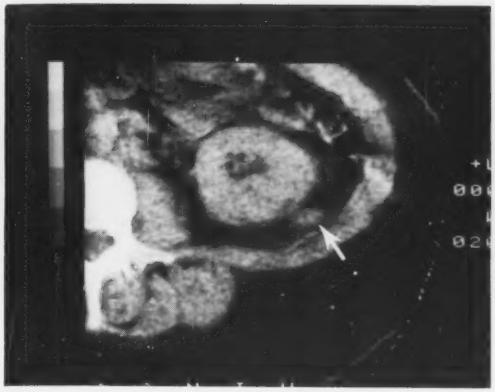

FIGURE 25.—CT scan performed in a 42-yr-old woman who remained asymptomatic after percutaneous renal biopsy. A small posterior perirenal hematoma is defined (→).

FIGURE 26.—46-yr-old man with lymphoma. A huge pre- and para-aortic mass is defined with direct invasion of the left renal hilum and associated hydronephrosis.

FIGURE 27.—26-yr-old man with a history of liver trauma. A large, well-defined posttraumatic hepatic cyst is present.

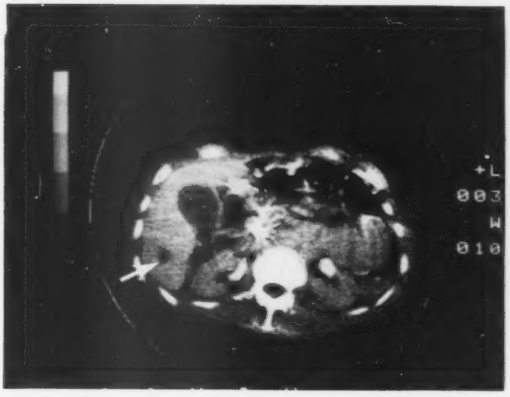

FIGURE 28.—Incidental visualization of a small hepatic cyst (→) measuring approximately 1 cm in dimension in a 46-yr-old woman with a pseudocyst in the head of the pancreas (◊).

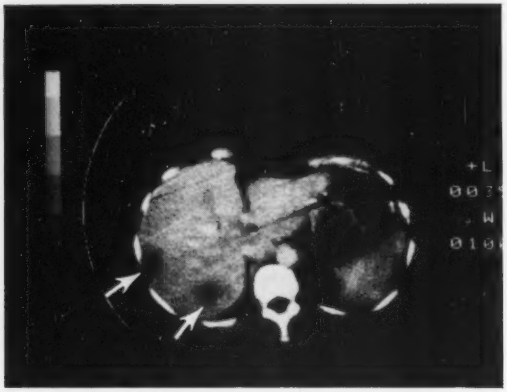

FIGURE 29.—58-yr-old woman with rectal carcinoma. Multiple metastases (→) are evident in the right lobe of the liver.

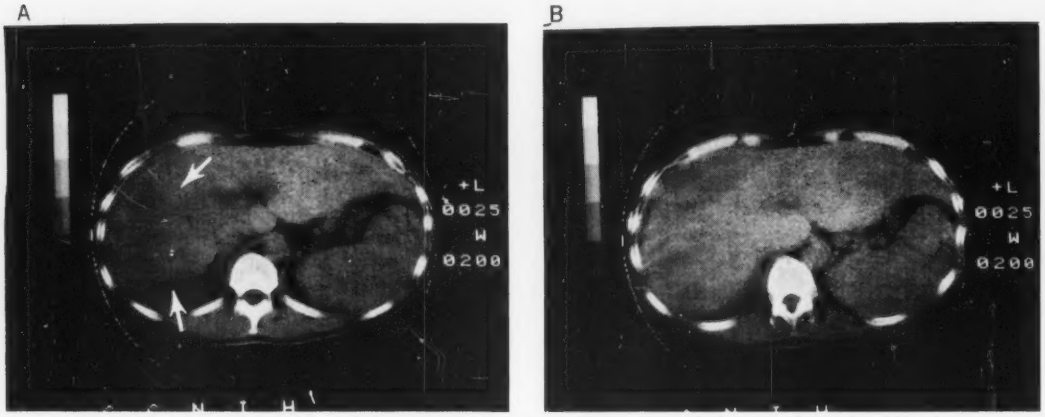

FIGURE 30.—44-yr-old man with hepatoma. (A) A large mass is noted occupying the right lobe of the liver (→). (B) It is not visible following contrast infusion.

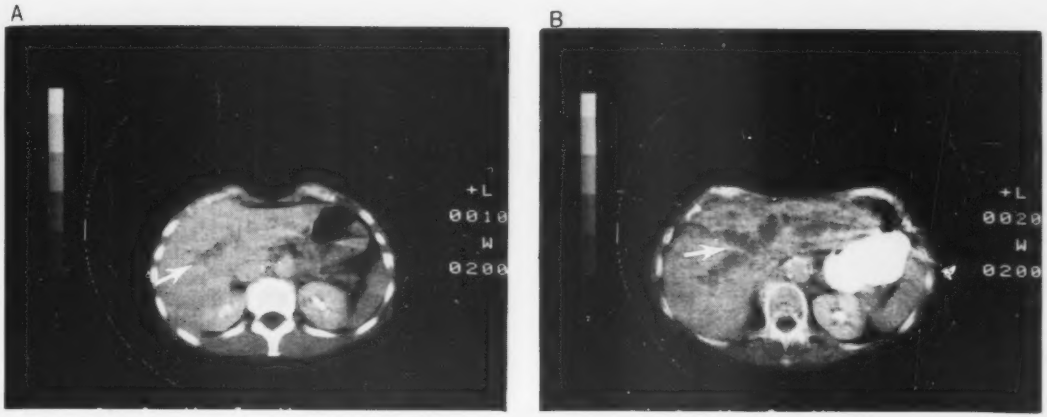

FIGURE 31.—(A and B) 2 patients with pancreatic carcinoma demonstrating dilated intrahepatic ducts (→) secondary to portal biliary obstruction.

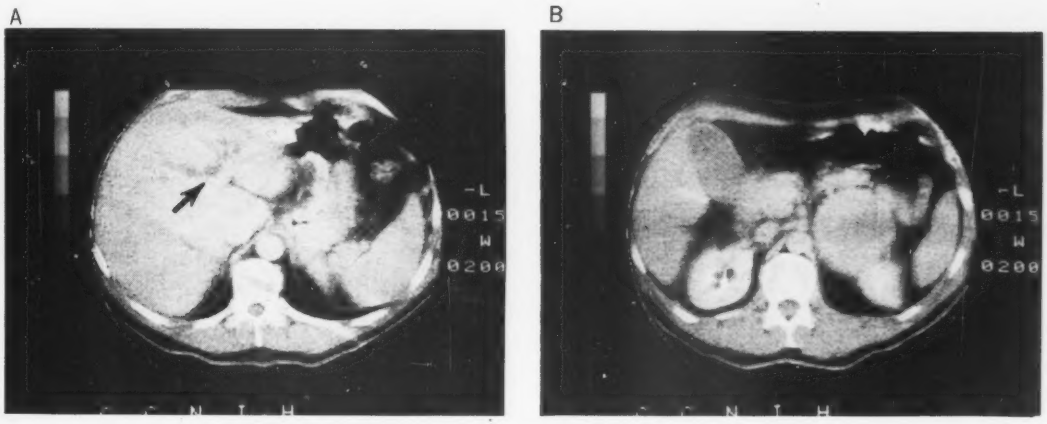

FIGURE 32.—34-yr-old man with melanoma. (A) Mild central hepatic duct dilation (→) and (B) enlargement of the gallbladder were incidentally noted secondary to nodal involvement in the porta. The patient subsequently developed clinical jaundice.

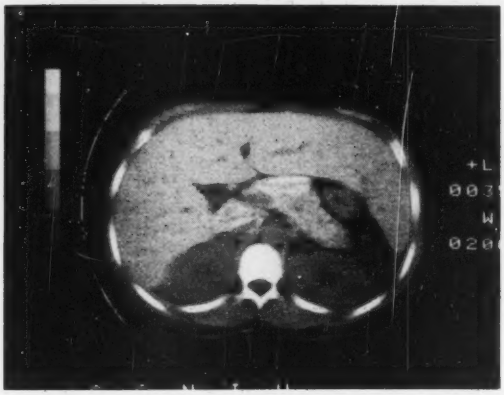

FIGURE 33.—21-yr-old man with hemochromatosis. The hepatic parenchyma is dense by virtue of its high iron content. Also note iron deposition throughout the pancreas.

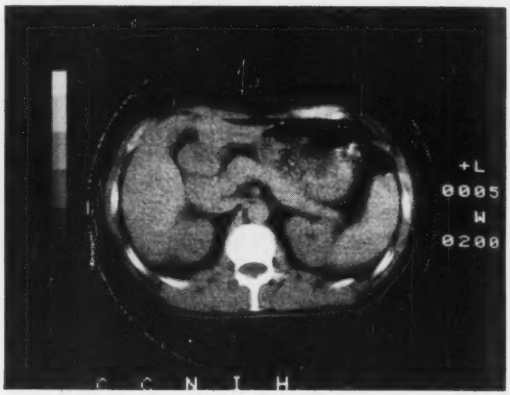

FIGURE 34.—CT scan demonstrating a normal transverse pancreas.

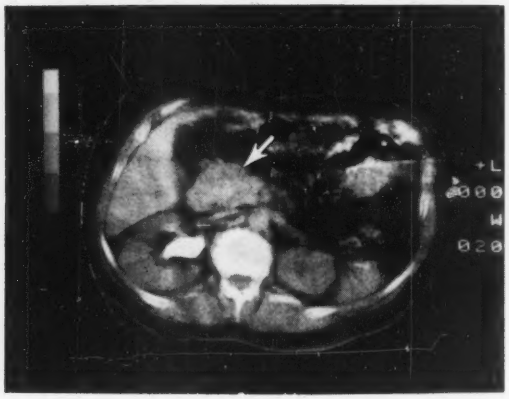

FIGURE 35.—61-yr-old woman with pancreatic carcinoma. A mass enlarging the pancreatic head is evident (→). Also note staghorn calculus in the right kidney.

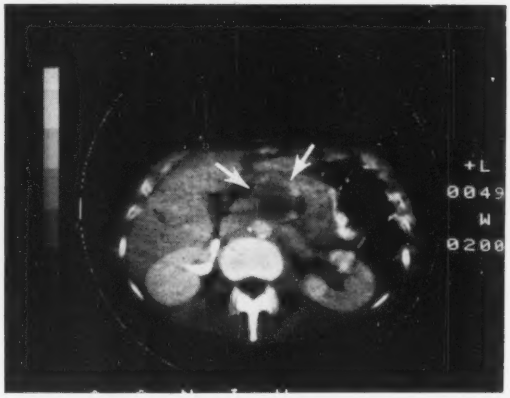

FIGURE 36.—51-yr-old man with carcinoma of the pancreas. A centrally necrotic mass (→) is defined in the body of the pancreas.

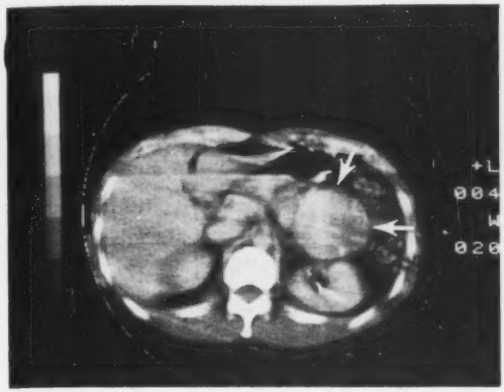

FIGURE 37.—31-yr-old man with suspected insulinoma. CT scan defines a mass in the tail of the pancreas subsequently confirmed to represent a malignant insulinoma.

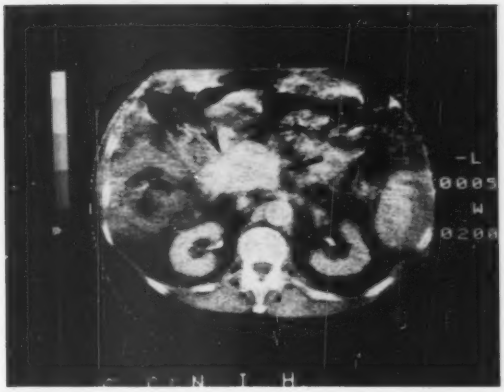

FIGURE 38.—54-yr-old man with Zollinger-Ellison syndrome. A mass (→) which enhanced following iv contrast infusion is present in the head of the pancreas. It proved to be a non-B-islet cell tumor.

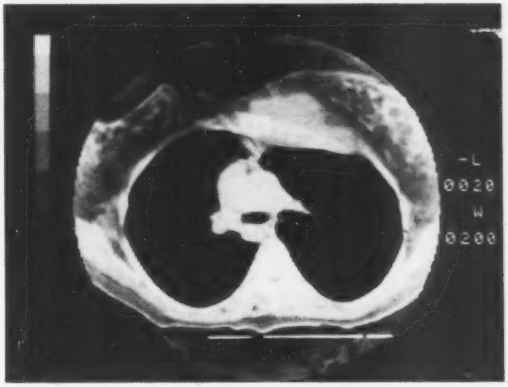

FIGURE 39.—16-yr-old girl with rhabdomyosarcoma of the chest wall. The intra- and extra-thoracic extent of the tumor is defined by CT. Pericardial extension was also present.

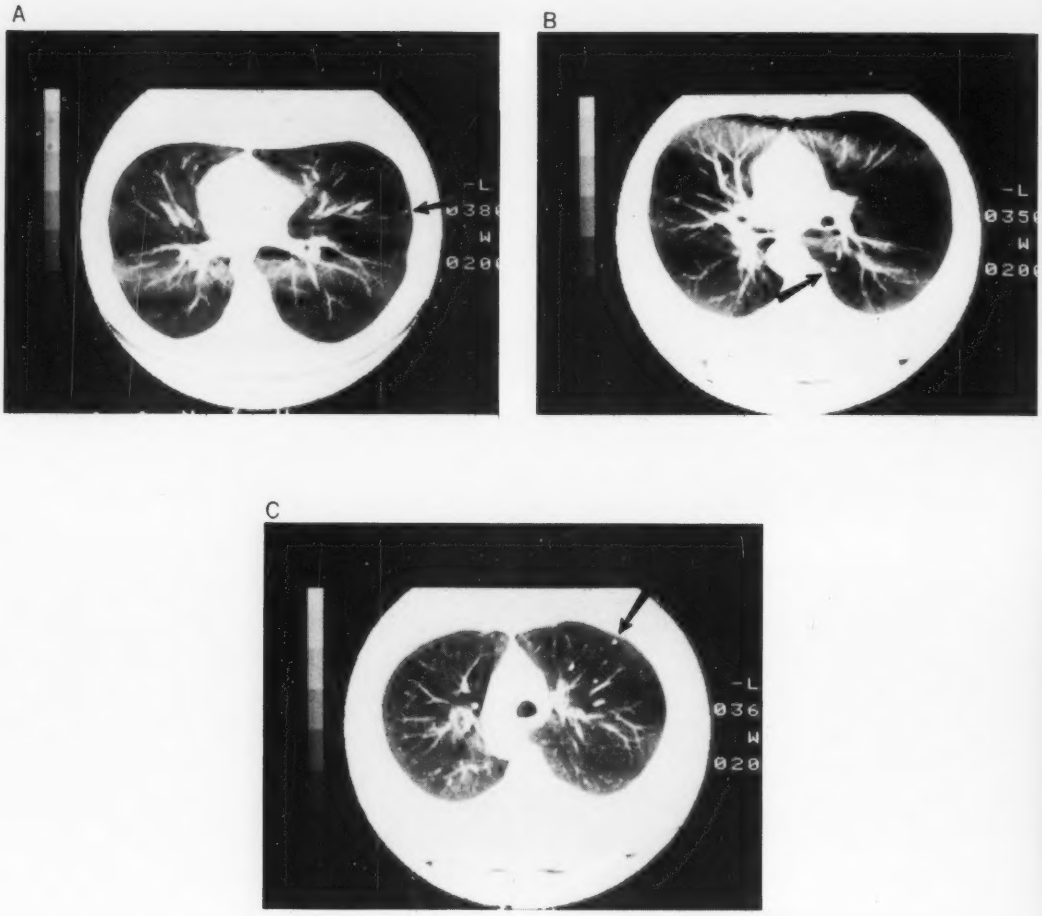

FIGURE 40.—3 patients (A, B, and C, respectively) in whom full lung CT of the chest defined additional pleural and subpleural nodules (→) not evident on standard tomograms.

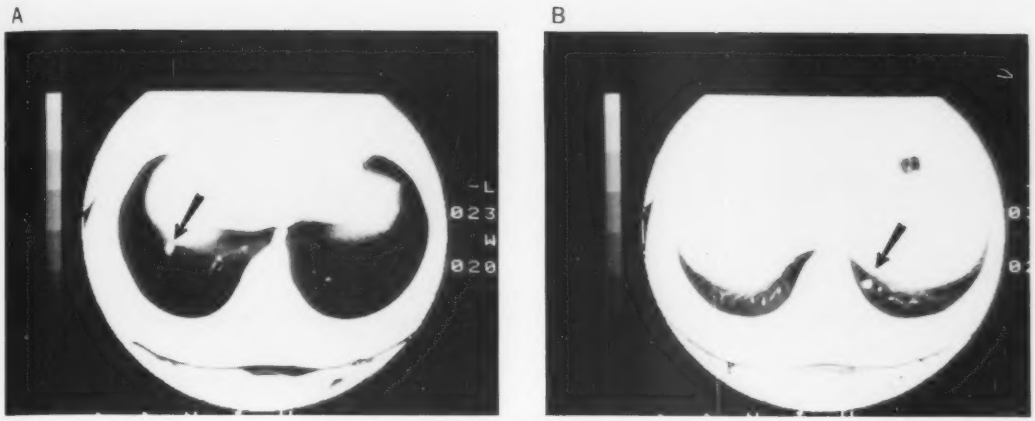

FIGURE 41.—18-yr-old man (A and B) with osteogenic sarcoma. CT defined bilateral metastases (→). Lesion in the left posterior gutter (B) which measured 1 cm in dimension was not evident even in retrospect on conventional tomography.

FIGURE 42.—61-yr-old man with hyperparathyroidism. An anterior mediastinal mass (→) is present, confirmed at angiography, which was surgically proven to represent a parathyroid adenoma.

FIGURE 43.—61-yr-old man with oat cell carcinoma. A lytic metastasis is defined destroying the T4 vertebral body, transverse process, and posterior spinous process. Note extension into the spinal canal.

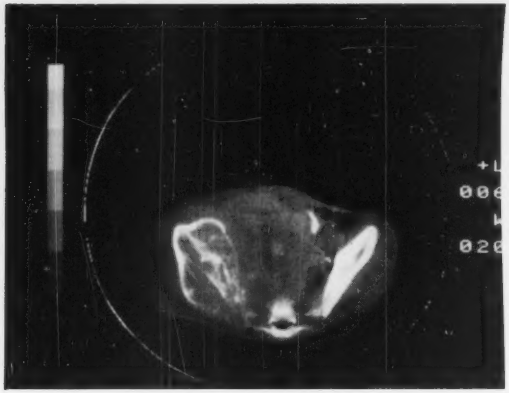

FIGURE 44.—12-yr-old boy with chondrosarcoma of the left iliac wing. Bone destruction and partially necrotic associated intra- and extra-pelvic soft tissue tumor extension is noted.

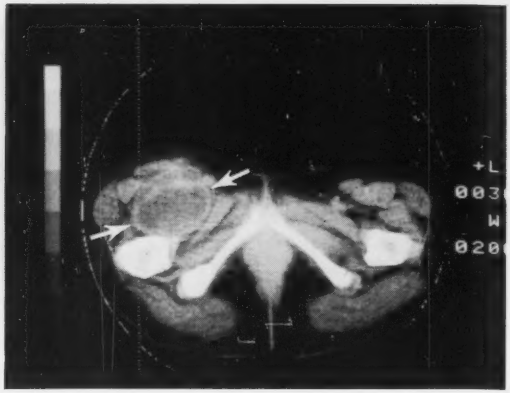

FIGURE 45.—40-yr-old man with a palpable right thigh mass. A lobulated mass of lower x-ray absorption than surrounding muscle groups is defined (→) which extended superiorly into the right hemipelvis and proved to be a liposarcoma at surgery.

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Observations on the Human Pharmacology of Cycloleucine [1,2,3]

Brief Reports and Preliminary Communications

Franco M. Muggia,[4,5,*] Jerome Loew,[4] and Susan Horwitz [4]

Cycloleucine (1-aminocyclopentane-1-carboxylic acid), a cycloaliphatic amino acid with experimental antitumor activity (1), was introduced into clinical trials in the late 1950s. The pharmacology of the compound has been extensively studied in mice and rats (2–4), and toxicologic information is available from both animal experiments (1) and clinical studies (5–12). On the other hand, human pharmacology data have not been generally available to clinical investigators. Based on observations of drug accumulation and excretion in one subject receiving daily doses of cycloleucine and on previously published pharmacologic studies in man (4,13), we wish to call attention to some important differences between the human and animal pharmacology of cycloleucine.

METHODS

Cycloleucine was measured by modifications of the method of Janssen et al (14). This involved oxidative decarboxylation and deamination of amino acids by ninhydrin and subsequent measurement by gas-liquid chromatography[6] of the cyclopentanone formed from cycloleucine.

Known amounts of cycloleucine were added to 2 ml of water, urine, and plasma and subjected to oxidation and chloroform extraction of the cyclopentanone to form appropriate standard curves. Ethylbenzene (10 ppm by volume) was added to the chloroform and internal standard. A 1-μl sample was chromatographed and the ratio of the peak area for cyclopentanone to that for ethylbenzene was obtained; peak areas were obtained directly from the integrator output. The ratio obtained for patient samples was compared to a standard curve of the ratios obtained from appropriate known solutions processed as above. Determinations of cycloleucine levels from heparinized plasma, cerebrospinal fluid (CSF), and urine of the subject were made using frozen samples obtained at appropriate times. Plasma samples were deproteinated prior to oxidation and extraction. Urine samples were diluted 1:10 with water prior to oxidation. Oxidation, extraction, and chromatography of a solution containing each of 19 naturally occurring α-amino acids in 10^{-3} M concentration showed that no products were formed which interfered with the chromatographic determination of either ethylbenzene or cyclopentanone.

CASE REPORT

A 19-year-old man underwent excision of an alveolar soft part sarcoma of the calf in December 1973, and was found to have bilateral pulmonary nodules. Treatment with cycloleucine was initiated as part of an Eastern Cooperative Oncology Group (ECOG) study comparing the effectiveness of this compound *versus* adriamycin in metastatic soft tissue sarcomas (12). In January 1974, the patient began receiving 4-hour infusions of 200 mg/kg (15 g) of cycloleucine, which were to be given monthly for 8 consecutive days. However, the first course was interrupted after four daily doses because the patient experienced headaches. Subsequent courses were also shortened to 3 or 4 days because of either thrombocytopenia or fever (39°C) and chills. The latter were most severe on the second day of drug administration but tests for possible contamination with pyrogens were negative (Penta J, personal communication). Samples for measurement of cycloleucine in plasma and urine were obtained before and during the third and fourth courses of therapy. The CSF sample was obtained during the fourth course. The tumor nodules showed no growth dur-

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[3]Cycloleucine was obtained from the Cancer Therapy Evaluation Program (CTEP), Division of Cancer Treatment (DCT), NCI, as a 40-mg/ml solution manufactured by Ben Venue Laboratories.

[4]Departments of Medicine (Dr. Muggia) and Pharmacology (Drs. Loew and Horwitz), Albert Einstein College of Medicine, Bronx, NY.

[5]*Present address:* CTEP, DCT, NCI, Bethesda, Md.

[6]Hewlett-Packard model 761A, equipped with integrator model 3370B.

Reprint requests to: Franco M. Muggia, MD, CTEP, DCT, NCI, Bldg 37, Rm 6A17, NIH, Bethesda, Md 20014.

ing this period; the patient remained asymptomatic and his weight was maintained at 75 kg. Cycloleucine was administered until disease progression became evident in September 1974.

RESULTS

Retention times generally ranged from 4.2 to 4.34 minutes for ethylbenzene and from 5.6 to 5.9 minutes for cyclopentanone. In any given day retention times varied within ± 0.02 minute on analysis of multiple samples.

Cycloleucine was detected in plasma or urine in concentrations as low as 12.5 μg/ml. The system was linear, at least through plasma concentrations of 400 μg/ml and through urine concentrations of 1600 μg/ml at 1:10 dilutions (equivalent to 16 mg/ml in undiluted urine). Duplicate samples gave results within 1.5%. Simultaneously obtained serum and heparinized plasma gave equivalent results.

The results of plasma drug levels indicated a range of 165 μg/ml, obtained 15 hours after completing the first-day infusion of cycloleucine, to a high of 390 μg/ml, 1 hour after completing the fourth day of drug administration. Urine levels increased from 2.3 g during the first-day infusion of 15 g to a high of 9.2 g obtained during the fourth day of the course. Urinary clearance of cycloleucine was calculated by using mean plasma concentrations of the drug obtained during the timed urine collections. These varied between 11.1 and 19.7 ml/minute with a creatinine clearance of 172 ml/minute.

The single determination in the CSF was made 1 hour after administering 10 g of cycloleucine on the final day of a 4-day course. The CSF concentration was 60 μg/ml while the simultaneous plasma level was 390 μg/ml. The plasma level had been maintained within 50% of this level for 20 hours preceding this determination. The calculated cumulative excretion of drug was 46.9% after 3 days in one course and 37+% after 4 days in another course (incomplete collection). One determination 13 days after completion of the course indicated 25 μg/ml of plasma concentration still remaining.

DISCUSSION

Animal studies have documented a plasma half-life of 9 and 22 days respectively in the mouse and rat (2,4). Two previous reports (4,13) have noted a much greater urinary excretion of cycloleucine in man than expected from the observations in animals. Although the data were incompletely reported in one study (13), both plasma and urinary decay rates in patients receiving doses of 300 mg/kg/day had a half-life of 2.4 days. After several days of drug administration, excretion in the urine approximated 90% of the daily dose. The other study (4) was performed with 1–2-mg amounts of ^{14}C-labeled cycloleucine. The half-life in plasma in two men, aged 33 and 46 years, was 3.2 and 3.8 days respectively; the cycloleucine clearance rate was 6.7 and 5.6 ml/minute. The higher clearance values found in our patient suggest that clearance may increase with higher plasma levels. Renal excretion in man is associated with a "cystinuria" type of aminoaciduria (13), which raises the question of a shared transport system between cycloleucine and cystine, ornithine, lysine, and arginine, resulting in defective tubular re-absorption of these diamino acids. This peculiar renal handling of cycloleucine may also explain its toxicologic differences, with the rhesus monkey tolerating much greater amounts relative to other animals (1).

Clinical studies have indicated a relatively increased human tolerance of cycloleucine. These started with oral or iv doses of 10–150 mg/kg/day given over 5–10-day periods (5–9) and were gradually escalated to doses of 200 mg/kg/day for 10 days (10) and 300 mg/kg/day for 8 days repeated monthly (11,12). The ECOG study initially adopted this schedule but later reduced it to 200 mg/kg after life-threatening CNS toxicity occurred in two patients.

The pharmacologic data in man point to a need for additional studies to (*a*) explore different dose schedules, and (*b*) establish the relationship of the dose-limiting acute toxicity to blood and CSF levels. If indeed CNS toxicity can be correlated with a range of peak blood levels, then more rational dose schedules can be designed which avoid the CNS toxicity by either maintaining subtoxic blood levels for long time periods (low-dose daily administration) or achieving peak levels repeatedly without accumulating neurotoxic concentrations of drug (high-dose weekly or biweekly administration). Hematologic effects or fever will be potential dose-limiting toxicities at new schedules.

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Preliminary Experiences With Intra-Arterial Adriamycin [1,2]

Brief Reports and Preliminary Communications

Pradeep Shah,* Laurence H. Baker, and Vainutis K. Vaitkevicius[3]

Adriamycin is an anthracycline antibiotic isolated from cultures of *Streptomyces peucetius* var. *caesius* (1). It has significant antitumor activity in a variety of neoplasms (2,3). Preliminary data suggest adriamycin traverses the cell membrane by active transport (4); it then induces a block of cell cycle progression in the G_2 phase. The degree of block may be a function of concentration and time (5). Hence, intra-arterial (IA) administration of adriamycin may be an ideal way to achieve maximum concentration of drug in tumor. Adriamycin is locally a vesicant, so it is essential to find the maximum safe concentration that can be infused. Haskell et al (6) used IA adriamycin in a 45–107-mg/m² dose as a continuous infusion over 1.5–3 days followed by surgery or radiotherapy. Di Pietro et al (7) used IA adriamycin at a dose of 0.3 mg/kg/day for 10 consecutive days and 14 mg/kg on alternate days for 10 days for a single cycle in each patient. This paper describes our preliminary experience with IA adriamycin used in multiple cycles to study local and systemic cumulative toxicity with various dose schedules.

MATERIALS AND METHODS

The patient population is shown in table 1. Adriamycin was dissolved in 40 ml of normal saline and infused at a constant rate over 6–8 hours with a Watkins or Howard pump. IA catheters were placed by standard surgical techniques. Angiograms were obtained in all cases. Dose schedules used in this study are outlined in table 2. An attempt was made to escalate dose or to increase frequency of cycles of treatment until the maximum safe dose or tumor response was reached. Five patients underwent dose escalation with the same schedule; all except one developed toxicity. Two patients were shifted to different dose schedules. In two cases the initial dose had to be decreased because of toxicities. Treatment was discontinued when definite progression of disease or no response was observed after two courses of treatment.

Patients were monitored with cbc and platelet counts weekly. When myelosuppression was detected, blood counts were done daily or on alternate days. Hepatic and renal functions were evaluated prior to each course of therapy. Tumor measurements were done before each course of treatment.

RESULTS

The toxic effects of the various dosage regimens are reported in table 2. It should be noted that three patients received > 500 mg/m² of adriamycin without any evidence of cardiomyopathy. Severe neutropenia with wbc counts < 1000/mm³ developed in two patients (Nos. 1 and 2) who had hepatic metastases infused through hepatic artery.

Partial objective response was documented in 50% of the patients. Responses lasted 3 weeks to 3 months with a median response of only 4 weeks. Both patients (Nos. 1 and 2) with hepatic metastasis, elevated bilirubin, and elevated alkaline phosphatase responded with a decrease in liver size, but the performance status did not improve and both developed severe neutropenia. Patient 1 (table 1) was treated with a daily continuous infusion of adriamycin at 10.7 mg/m²/day for 5 weeks without toxicity. After 2 weeks of no treatment, the patient was treated with continuous adriamycin infusion at an increased dose of 14 mg/m²/day for 5 more weeks. The patient developed severe protracted myelosuppression and severe mucositis. He recovered from drug toxicity but died of hepatic failure 4 weeks later.

Patient 2 (table 1) was treated with 25 mg/m²/day × 3 days by a 6–8-hour infusion of adriamycin. Neutropenia began on the 6th day and the nadir was reached at the 7th day with a recorded wbc count of 600/mm³. On the 16th day patient 2 recovered from myelosuppression. On the 21st day the second cycle was initiated at a decreased dose of 20 mg/m²/day for 3 days. He developed depression

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[3]Section of Clinical Pharmacology, Department of Oncology, Wayne State University School of Medicine, Detroit, Mich.

*Reprint requests to: Laurence H. Baker, DO, Wayne State University, Department of Oncology, Harper-Grace Hospitals, 4160 John R St, Detroit, Mich 48201.

TABLE 1.—Patient population

Patient No.	Patient age/sex	Diagnosis	Area involved	Artery infused	Dose schedule*	No. of cycles	Antitumor response†
1	69/M	Leiomyosarcoma, rectum (resected) with hepatic metastasis	Liver	Hepatic	III, IV	1, 1	PR
2	53/M	Adenocarcinoma, colon (resected) with hepatic metastasis	Liver	Hepatic	I	2	PR
3	15/M	Osteogenic sarcoma, maxillary sinus and orbital floor	Orbit	External carotid	VIII	2	Not evaluable
4	50/M	Rhabdomyosarcoma, orbit	Orbit	External carotid	I	4	PR
5	17/M	Fibrosarcoma, ear	External ear	External carotid	V	2	NR
6	66/M	Melanoma, thigh	Thigh	Common iliac	V, II	2, 6	PR
7	41/M	Lipofibrosarcoma, thigh	Thigh	Common iliac	I	3	PR
8	52/F	Cystosarcoma, phylloides, breast; metastasis, head and femur	Hip	Common iliac	I	3	PR
9	47/F	Lipomyxosarcoma, right thigh	Right thigh	Right common iliac	VII	1	NR
10	31/F	Chondrosarcoma, iliac bone	Hemipelvis	Internal iliac	I, V	1, 2	NR
11	18/F	Epithelioid sarcoma, sacrum	Pelvis	Lower abdominal aorta	VI, II	1, 7	PR
12	55/F	Liposarcoma, leg	Leg	Femoral	IX, II	3, 2	NR

*See table 2 for definitions.
†PR = partial response; NR = no response.

TABLE 2.—Dose schedules and toxic effects

Schedule No.	Dose schedule	No. of courses	Toxic effect						
			Local toxicity	Arterial thrombosis	Mucositis	Gastro-intestinal symptoms (nausea/vomiting)	Neutropenia (< 4000 cells/mm³)	Thrombo-cytopenia (< 100,000 cells/mm³)	Alopecia
I	20–30 mg/m²/day × 3 days, q3wks*	13	0	0	1	3	4	2	13
II	75–140 mg/m²/wk*	15	1	1	0	0	0	0	15
III	10.7 mg/m², continuous daily infusion × 5 wks	1	0	0	0	0	0	0	1
IV	14 mg/m², continuous infusion × 5 wks	1	0	0	1	1	1	1	1
V	75 mg/m², q3wks*	6	0	1	1	1	0	0	6
VI	75 mg/m²/day × 7 days*	1	0	0	0	0	0	0	1
VII	40 mg/m²/day × 4 days*	1	0	0	0	0	0	0	1
VIII	15–20 mg/m²/day × 5 days, q3wks*	2	2	0	2	1	2	0	2
IX	25–45 mg/m²/wk*	3	0	0	0	0	0	0	2

*Daily dose was a 6–8-hr infusion.

of wbcs for 10 days with the lowest wbc count being 1900/mm³ on the 8th day of the second cycle. Patient 2 died of hepatic failure 5 weeks after the second course.

Two patients had IA adriamycin as primary treatment followed by surgery and radiation therapy respectively, and both are alive 3 years later without recurrence. Patient 9, a 47-year-old white woman, had liposarcoma of the right thigh. She received regional infusion of adriamycin (40 mg/m²/day × 3 days) followed by radical radiation to the right thigh and hemipelvis. She is alive after 4½ years without any evidence of recurrence. Patient 12, a 55-year-old white woman with liposarcoma of the left leg, refused surgery. Initially, she received IA adriamycin at a dose of 25–125 mg/m²/week given as a continuous infusion for five cycles without objective regression of tumor. She finally agreed to have an above-knee amputation. She is alive without recurrence after 3½ years.

DISCUSSION

It appears that IA adriamycin at a dose of 20–30 mg/m²/day for 3 days repeated every 3 weeks is a relatively safe schedule. In four of nine reported schedules only a single course of therapy was given and hence these regimens are not evaluable. No local tissue reaction of the infused artery was noted. Systemic toxicity is also limited with only one episode of mucositis and moderate neutropenia in four of 13 treatments. Higher doses of 75–140 mg/m²/week are not considered to be safe because of marked inflammatory and necrotic local reaction of perfused tissue. In two cases hepatic metastasis was treated with hepatic artery infusion of adriamycin. Both patients had severe myelosuppression. This clinical experience is consistent with the known pharmacokinetics of adriamycin in man (8,9).

In ten advanced cases IA adriamycin was used with intent of palliation. More than 50% regression of measurable lesion was noted in five. There were no drug-related deaths. In conclusion, IA adriamycin can be given safely and effectively at a dose of 20–30 mg/m²/day in 6–8-hour infusions repeated every 3 weeks. Its place in palliation of cancer is questionable, but as part of combined chemotherapy, surgery, and radiotherapy IA adriamycin may be of potential benefit.

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Effect of Sequence of Combined Radiation and Cyclophosphamide Regimens on Murine Osteosarcoma [1,2]

Brief Reports and Preliminary Communications

Anas M. El-Mahdi* and James Shaeffer [3]

The multimodal use of radiotherapy and chemotherapy in the treatment of malignant neoplasms, especially disseminated disease, is increasing. The sequence in which these agents are administered, that is, drug first, radiation first, or concurrent drug and radiation, is often decided empirically. The lack of a fundamental understanding of the added toxicities of these agents has sometimes resulted in deaths (1–4). Reports of interactions between oncotherapeutic drugs and radiation are not uncommon (4–7) as these drugs can augment the toxic effects of radiation in normal tissues as well as in tumors.

Using a mouse model in which Dunn osteosarcoma cells are injected iv into C3H/HeDub recipient mice to form pulmonary tumor colonies, we have previously shown thoracic irradiation and cyclophosphamide (CP) as single agents to be effective in eradicating pulmonary colonies and prolonging survival (8,9). This investigation was designed to determine how the sequence of administration of radiation and CP in a multimodal approach might affect the efficacy of the treatment.

MATERIALS AND METHODS

Details regarding the mice, tumor, cell suspension preparation, and pulmonary colony assay have been published elsewhere (8–10). The Dunn osteosarcoma is a transplantable tumor of spontaneous origin (11). It is maintained by serial sc transfer at monthly intervals in female C3H/HeDub mice (obtained from Flow Research, Dublin, Va).

For pulmonary colony production, iv injections of single-cell suspensions prepared from sc grown tumors were used. Tumor inocula were adjusted to produce about 50–100 colonies per mouse in untreated control animals. All radiation and CP treatments were begun 14 days after tumor cell injection. Mice were 10–12 weeks old at this time. Tumor colonies at 14 days are visible to the naked eye, with an average volume of about 1×10^{-2} mm^3 (8–10).

Mice given radiation were first anesthetized with 0.065–0.070 mg/g of sodium pentobarbital ip. Single anterior ^{60}Co fields were used. Mid-thoracic doses (80-cm source tumor distance) were either 1750 or 1000 rads, the anterior to posterior variation in dose being about ± 5%, as determined by thermoluminescent dosimetry with lithium fluoride chips. A 2.5 × 23.0-cm portal was used to irradiate groups of six to eight mice; the dose rate was about 135 rads/minute.

CP treatments consisted of the ip injection of 50 mg/kg/day for 3 consecutive days. This dose is below the lethal threshold of 100 mg/kg/day × 3 (LD50 = 180 mg/kg/day × 3 [8]). In those groups receiving combined treatments, a 1-, 3-, or 7-day interval interposed the two treatments. After treatment, the mice were randomly divided into two groups: one group was killed 30 days after treatment (44 days after tumor cell injection) for lung colony counts and the other group was kept for survival studies. Mice in the latter groups were weighed weekly as an overall indication of health.

Untreated control animals that died before 44 days after tumor cell injection were scored for lung colony counts provided they died ≥ 30 days after tumor cell injection, after which time lung colony appearance plateaus (8). The extent of tumor control in the treated groups was calculated as follows: 1–(mean tumor colonies in treated group ÷ mean tumor colonies in control group).

Peripheral wbc counts were performed using an electronic counter.[4] Tips of the tails (2–3 mm) were cut off and about 50 μl of whole blood was collected. Silver nitrate sticks were used to cauterize the wounds. Mice in the blood count groups were not used for lung colony analysis or survival studies.

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[3]Department of Radiation Oncology and Biophysics, Eastern Virginia Medical School, Norfolk.

*Reprint requests to: Anas M. El-Mahdi, MD, ScD, Medical Center Hospitals, 600 Gresham Dr, Norfolk, Va 23507.

[4]Coulter Electronics, Hialeah, Fla.

TABLE 1.—Tumor control and survival in mice treated with radiation and/or CP 14 days after iv injection of Dunn osteosarcoma*

Treatment group	No. of mice killed	Mean lung colonies ± SE	% tumor control	Survival 60 days after treatment
Control	28	49.9 ± 6.4	0	0/38
Radiation	19	17.9 ± 2.3	64	2/20 (10%)
CP	19	4.3 ± 0.9	91	9/20 (45%)
Radiation, 7 days' rest, CP	19	1.1 ± 0.3	98	4/20 (20%)
CP, 7 days' rest, radiation	19	0.50 ± 0.19	99	3/16 (19%)

*Radiation treatments were a single thoracic dose of 1750 rads; CP treatments were 50 mg/kg/day ip × 3 days.

RESULTS

Tumor control and survival of mice given CP and 1750 rads alone or in combination are summarized in table 1. Although neither agent alone was lethal in tumor-free mice over a 60-day observation period, the combination of the two proved lethal. A group of 22 tumor-free mice given 1750 rads, a 7-day rest, and CP had a mean ± SD lifespan of 57.7 ± 19.9 days (range, 21–93 days). Likewise, in a group of 17 tumor-free mice the CP, 7-day rest, 1750-rad combination resulted in a 57.9 ± 18.3-day mean lifespan (range, 32–90 days).

For this reason, experiments were repeated using a lower radiation dose (1000 rads) combined with the CP. Tumor-free mice given 1000 rads, 7-day rest, and CP had a 92% 90-day survival rate (22 of 24 mice), while the reverse sequence resulted in a 67% survival rate (16 of 24 mice).

Lung colonies and animal survival of tumor-bearing mice given various combinations of 1000 rads and CP are summarized in table 2. Radiation-first regimens resulted in general weight loss whereas mice given CP first gained weight. By 7 weeks after treatment, the three radiation-first groups had average weight losses ranging from 0.2 to 0.8 g while the corresponding CP groups gained 1.7–2.3 g.

Mice given a thoracic dose of 1000 rads had 72%, 51%, and 61% of control wbc counts at 1, 3, or 7 days after treatment respectively. Corresponding values for mice given CP were 53%, 83%, and 124%. These values are averages for six mice per group. The absolute leukocyte counts (mean ± SD) before treatment in the control, radiation, and CP groups were 9533 ± 838/mm^3, 9917 ± 3092/mm^3, and 9850 ± 1257/mm^3 respectively.

DISCUSSION

The combination of 1750 rads with the CP treatment did not appreciably improve the results obtainable by CP alone. There was an insignificant increase in tumor control and a decrease in survival in both combination treatment groups (table 1). The decrease in survival most probably resulted from the added burden of two agents. Indeed, these combinations of 1750 rads and CP were fatal in tumor-free mice although each agent alone was nonlethal over a similar 60-day observation period.

The combination of 1000 rads with CP did not significantly improve the level of tumor control obtained by CP alone (table 2). The three CP-first combination treatments had significantly longer survival ($P < 0.005$ by chi-square analysis) than the three radiation-first combinations, although none of the combination groups had significantly greater survival than that for CP alone. In addition to longer survival, the CP-first combination treatments seemed to be better tolerated in terms of weight gains compared to the radiation-first combination treatments. From table 2 it appears that animal survival is not directly related to the degree of tumor control, but depends on other host factors such as toxicity to normal tissues.

CP itself is inactive and is biotransformed into active alkylating metabolites by microsomal enzymes primarily in the liver and to a lesser extent in the lungs (12). Both radiation (13,14) and anesthetic barbitals (15) can modify the activity of microsomal enzymes. Thoracic irradiation in the mouse includes some of the liver directly in the field, as well as scattered radiation to that part of the liver not included in the field.

Microsomal enzyme modifiers have been shown to alter the toxic effects of CP in mice without changing its therapeutic effect (16). These findings suggest that the toxic and oncotherapeutic effects of CP may occur by different mechanisms. Our data with 1000 rads and CP support this hypothesis. The sequence of the two agents did not significantly change the degree of tumor control, although the CP-first groups had significantly higher survival at 90 days than did the radiation-first groups. Both the weight gains and higher wbc counts in the CP-first groups are consistent with their > 90-day survival. The lower toxicity of the CP followed by

TABLE 2.—Tumor control and survival in mice with 14-day Dunn osteosarcoma pulmonary colonies treated with various radiation and CP regimens*

Treatment group	No. of mice killed	Mean lung colonies ± SE	% tumor control	Survival 90 days after treatment
Control	25	109.9 ± 7.9	0	0/32
Radiation	12	82.3 ± 14.9	25	0/20
CP	22	13.8 ± 3.7	87	6/20 (30%)
Radiation, 1 day's rest, CP	16	5.4 ± 2.0	95	2/16 (12%)
Radiation, 3 days' rest, CP	14	13.8 ± 4.9	87	1/16 (6%)
Radiation, 7 days' rest, CP	14	16.7 ± 3.7	85	0/16
CP, 1 day's rest, radiation	16	9.5 ± 3.1	91	16/32 (50%)
CP, 3 days' rest, radiation	16	11.8 ± 4.2	89	8/32 (25%)
CP, 7 days' rest, radiation	16	1.8 ± 0.6	98	7/32 (22%)

*Radiation treatments were a single thoracic dose of 1000 rads; CP treatments were 50 mg/kg/day ip × 3 days.

radiation schedules may be related to the more rapid leukocyte recovery following CP treatment compared to radiation. Similar results have been obtained in other laboratories (15,17).

Further studies using different animal tumor models as well as different antitumor drugs in combination with radiation should provide more insight into the question of which agent should come first. The Virginia Oncology Group is currently planning a prospective study on the effect of sequence of radiation and CP in patients with oat cell carcinoma.

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5-FU Infusion and Cyclophosphamide in the Treatment of Advanced Ovarian Cancer [1,2]

Brief Reports and Preliminary Communications

Ronald M. Izbicki,* Laurence H. Baker, Michael K. Samson, Bruce McDonald, and Vainutis K. Vaitkevicius [3]

Although single-agent chemotherapy with an alkylating drug remains the treatment of choice for advanced ovarian cancers, polychemotherapy is increasing in popularity because of reported increases in response rates and survival when compared to single agents. Combination chemotherapy has been reported superior to single drugs in the treatment of ovarian cancer (1). The rationale of combination chemotherapy has been to combine drugs that are known to be effective as single agents in a particular tumor. Since most anticancer agents cause myelosuppression, the doses of these drugs used in combination must often be significantly reduced. Based on observations in our institution, 5-fluorouracil (5-FU), when given as a continuous infusion, had mucositis rather than myelosuppression as its dose-limiting toxicity (2). Thus, combining large doses of 5-FU (by infusion) with full doses of cyclophosphamide became feasible. 5-FU, in addition, has been reported to be active in the treatment of advanced ovarian carcinoma (3). In April of 1974 we undertook a study of 96-hour continuous infusion of 5-FU and full doses of cyclophosphamide.

MATERIALS AND METHODS

A total monthly dose of 5-FU by infusion was 4000 mg/m². A dose of 1000 mg/m² of 5-FU was given in 1000 ml of 5% D/W and infused over 24 hours. This was done daily for 4 days. The total monthly dose of cyclophosphamide was 1600 mg/m² for good-risk and 1200 mg/m² for poor-risk patients. The appropriate monthly dose of cyclophosphamide was administered as an iv bolus of 400 mg/m² daily for 3 or 4 days. A poor-risk patient is defined as a patient who is > 65 years old or has had > 3000 rads of pelvic radiation. Subsequent doses were adjusted according to toxic effects.

Wbc and platelet counts were obtained weekly and SMA-17 determinations were done before treatment and monthly thereafter. Tumor measurements were also recorded before treatment and then monthly. Complete objective regression was defined as disappearance of all measurable disease (a planned second look and laparoscopies were not part of the original study design). Partial regression was defined as 50% reduction of all measurable disease. The results of this study were compared to a historical control of 96 consecutively treated patients who received cyclophosphamide alone at our institution. The dose of cyclophosphamide in the control group was 1600 mg/m² for good-risk and 1200 mg/m² for poor-risk patients, repeated monthly.

The median age of the study patients was 60 years (range, 31–78 years) while the median age of the control group was 61.2 years (range, 20–74 years).

There were 29 evaluable patients in the treated group. One patient was lost to followup. There were 89 evaluable patients in the control group, three early deaths (before 2 weeks), and four lost to followup.

The histologies in both groups were comparable. An equal percentage of patients with ascites, bowel obstruction, and extra-abdominal disease were present in both groups. The performance status for the treatment group was a median of 60 (range, 20–80); the median for the cyclophosphamide group was 70 (range, 30–90).

Twenty-nine of 96 cyclophosphamide-treated patients and five of 30 5-FU-cyclophosphamide patients had had previous abdominal or pelvic radiation. Six of the cyclophosphamide-alone patients and none of the 5-FU-cyclophosphamide patients had had previous chemotherapy.

TOXICITY

A summary of toxicity is shown in table 1. Hematologic toxicity is further delineated in table 2. The nadir of leukopenia occurred at Day 6 with recovery by Day 9.

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[3]Department of Oncology, Wayne State University School of Medicine, Detroit, Mich.

*Reprint requests to: Ronald Izbicki, DO, Department of Oncology, Wayne State University School of Medicine, 4160 John R St, Detroit, Mich 48201.

TABLE 1.—Toxic effects

Toxic effect	No. of patients (%)	
	5-FU-cyclophosphamide	Cyclophosphamide
Hematologic*	22(76)	54(61)
Mild	6(27)	31(58)
Moderate	12(55)	19(35)
Severe	3(14)	4(7)
Life-threatening	1(4)	0(0)
Nausea and vomiting	21(72)	36(40)
Stomatitis	12(41)	21(30)
Hair loss	18(62)	31(46)
Thrombophlebitis	14(49)	0(0)
Sepsis	3(10)	0(0)
Cystitis	4(8)	14(16)
Total No. of patients treated	29	89

*Degree of hematologic toxicity =	Wbcs × 10^3/mm^3	Platelets × 10^3/mm^3
Mild	3.0–3.9	75–99
Moderate	2.0–2.9	50–74.9
Severe	1.0–1.9	25–49.9
Life-threatening	<1.0	<25

TABLE 2.—Hematologic toxicity

	Leukopenia (× 10^3 cells/mm^3)	Thrombocytopenia (× 10^3 cells/mm^3)
Mean	2.1	134
Median	1.8	110
Range	0.2–9.8	30–460

CLINICAL RESPONSE

Of the evaluable patients, the remission rate for the combination-treated group was 20 of 29 (68.9%) versus 40 of 89 (45.4%) for the cyclophosphamide-treated group ($P = 0.02$). Of the 20 remissions in the combination-treated population, nine were complete (31%) versus ten of 89 evaluable patients (11.3%) in the cyclophosphamide group ($P = 0.009$).

Of the ten patients who achieved partial remission, the median time from the start of therapy to maximum remission was 1.6 months (range, 1–3 months). The median time from the start of therapy to complete remission was 3.2 months (range, 2–4 months).

Poor patient compliance or thrombophlebitis necessitated the discontinuation of 5-FU infusion. The median number of monthly cycles of 5-FU infusion-cyclophosphamide given was 5.3. The largest number of monthly courses was 14. After 5-FU was discontinued, patients were maintained on cyclophosphamide alone.

The median duration of complete remission in both the 5-FU and cyclophosphamide-treated patients and the cyclophosphamide-alone group was 18 months.

A statistically longer survival was seen for the 5-FU infusion-cyclophosphamide-treated patients when compared to patients treated with cyclophosphamide alone ($P < 0.05$) (*see* fig 1). The median survival for the study group was 13.5 months versus 7.5 months for the cyclophosphamide-treated group.

After tumor progression occurred, there were ten of 89 (11.3%) cyclophosphamide-treated patients who achieved partial remission after subsequent therapy; no patient in the combination-treated group responded to other agents after tumor progression was noted.

DISCUSSION

We have demonstrated the ability to give full doses of two active agents in the treatment of advanced ovarian cancer. This combination produced a near tripling of the complete response in comparison to our historical controls. This increase in response rate appears to be reflected by improved

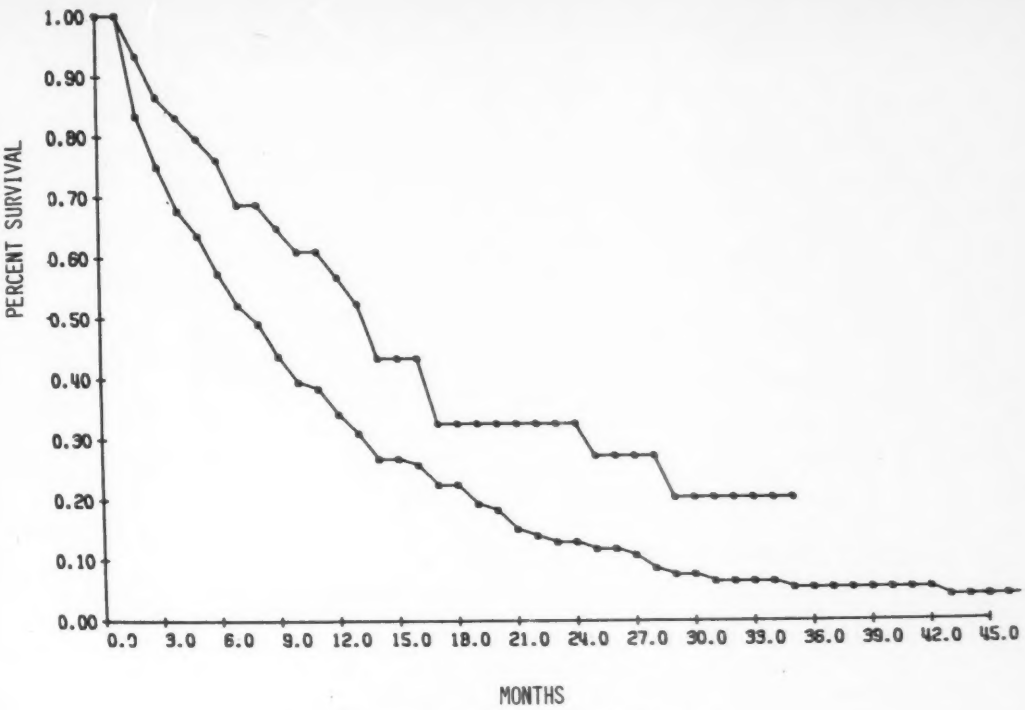

FIGURE 1.—Survival of all patients from the start of treatment; □-□-□ = cyclophosphamide and ○-○-○ = 5-FU-cyclophosphamide.

survival of the patients treated with 5-FU and cyclophosphamide. Although the treatment and control group appear comparable, a randomized clinical trial may have eliminated less obvious differences in the two treatment groups.

The involved nature of 5-FU infusion makes the treatment more cumbersome than cyclophosphamide alone. Nonetheless, we feel this combination therapy deserves further therapeutic trial and may be of value as induction therapy to reduce tumor burden.

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A New Antineoplastic Amino Acid Derivative, A-924 [1,2]

Haruhisa Fujita,[3,*] Toshiharu Sakurai,[4] Hiroko Ichimura,[4] Hiroshi Sato,[4] and Shigeshi Toyoshima [3]

In a previous report, Fukushima and Toyoshima described five amino acid derivatives that were found to be highly effective against Ehrlich ascites tumor and some other tumor types (1). Further experiments were performed to find an orally administratable amino acid derivative which possesses low toxicity and a specific antitumor spectrum. A-924 [2,8-di-*N*-bis-(*n*-butyloxycarbonylaminomethyl)-L-citrulline] was found to be highly effective against several ascites hepatomas when administered orally. This report includes results obtained on the antineoplastic effects of A-924 against animals implanted with ascites hepatomas.

MATERIALS AND METHODS

The chemical structure of A-924 is shown in figure 1. A-924, suspended in 0.5% carboxymethylcellulose as the vehicle, was administered at 1.00 ml/kg of body weight. Male Donryu rats, weighing 150–200 g, were used. The effect on life prolongation was determined from experiments conducted on the following ascites hepatoma cell lines: AH 13, AH 44, AH 66, AH 66F, AH 130, AH 272, AH 7974, AH 109A, AH 41C, and AH 60C. To confirm the antitumor activity and the specific antitumor spectrum of A-924 against ten rat ascites hepatomas, the following three therapeutic experiments were conducted. Experiment 1 rats were inoculated ip with 1×10^7 cells of tumor on Day 0; A-924 was administered ip, 300 mg/kg/day, for 10 consecutive days beginning on Day 3 after tumor implantation. Experiment 2 rats were inoculated iv with 1×10^7 cells of tumor on Day 0; A-924 was administered ip, 300 mg/kg/day, for 10 consecutive days beginning on Day 3 after tumor implantation. Experiment 3 rats were inoculated iv with 1×10^7 cells of tumor on Day 0; A-924 was administered orally, 300 mg/kg/day, for 10 consecutive days beginning on Day 3 after tumor implantation.

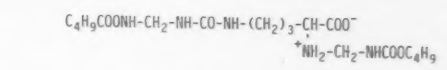

FIGURE 1.—Chemical structure of A-924.

RESULTS AND DISCUSSION

Antitumor effects determined by the three different therapeutic models are summarized in table 1. As can be seen in table 1, A-924 showed broad antitumor spectrum when given ip against the ip implanted model. Moreover, A-924 produced significant prolongation of the survival time of rats bearing ascites hepatoma such as AH 13, AH 66, AH 130, AH 41C, and AH 66F, when given ip or orally against iv implanted models. These results suggest that A-924 shows a higher antitumor activity and possesses a broader antitumor spectrum when given ip or orally against an iv implanted model than when administered ip against ip implanted models. Ichimura reported that among ten types of rat ascites hepatoma, AH 60C in particular exhibited complete resistance against all available antitumor drugs (2) and AH 41C was insensitive against almost all available antitumor drugs except procarbazine when given orally against an iv implanted model (3). The degree of drug resistance of these ten ascites hepatomas was as follows: AH 60C > AH 41C > AH 7974 > AH 66F > AH 109A > AH 272 > AH 44 > AH 60 > AH 130 > AH 13 (4). Particular interest was paid to the activity of A-924, given orally, against AH 41C implanted iv in rats. To clarify the anti-AH 41C effect of A-924 when given orally against an iv implanted model, the minimum effective oral dose of A-924 was determined against rats implanted iv with AH 41C. The results obtained are shown in table 2.

These results indicate that while orally administered minimum doses of 100 mg/kg/day definitely

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[2]A-294: 2,8-di-*N*-bis-(*n*-butyloxycarbonylaminomethyl)-L-citrulline.

[3]Division of Chemotherapy, Pharmaceutical Institute, Medical School of Keio University, Shinanomachi, Shinjukuku, Tokyo, Japan.

[4]Department of Pathology, Sasaki Institute, Kanda Surugadai, Chiyodaku, Tokyo, Japan.

*Reprint requests to: Dr. Haruhisa Fujita, Division of Chemotherapy, Pharmaceutical Institute, Medical School of Keio University, Shinanomachi, Shinjukuku, Tokyo 160, Japan.

TABLE 1.—Antitumor activity of A-924 on survival time in rats bearing various ascites hepatoma lines

Tumors of ascites hepatoma	Experiment 1*		Experiment 2*		Experiment 3*	
	No. of survivors/ total No. of rats†	Increase in lifespan (%)	No. of survivors/ total No. of rats†	Increase in lifespan (%)	No. of survivors/ total No. of rats†	Increase in lifespan (%)
AH 13	0/10	40‡	0/10	20	4/10	154§
AH 66	0/10	60‡	5/10	159§	0/10	23
AH 130	0/10	30	2/10	80¶	2/10	66¶
AH 7974	2/10	81¶	2/10	80¶	0/10	30
AH 41C	0/10	81¶	5/10	115§	7/10	169§
AH 44	0/10	22	7/10	175§	8/10	185§
AH 66F	0/10	80¶	2/10	148§	2/10	131§
AH 272	0/10	78¶	0/10	25	0/10	16
AH 109A	0/10	13	0/10	67	0/10	15
AH 60C	0/10	47‡	0/10	29	0/10	32

*See Materials and Methods for evaluation of antitumor activity grade.
†On Day 60 after tumor implantation.
‡$P < 0.05$.
§$P < 0.001$.
¶$P < 0.01$.

TABLE 2.—Effect of A-924 on survival time of rats implanted iv with AH 41C ascites hepatoma

Experimental group	Dose* (mg/kg)	No. of survivors/ total No. of rats	Mean survival time (days ± SD)	Increase in lifespan (%)
Tumor control†	—	0/10	20.6 ± 2.65	—
A-924	100	2/10	31.0 ± 9.80‡	50.5
	200	4/10	39.7 ± 17.4§	92.7
	300	7/10	52.9 ± 16.7¶	156.7

*Drug was administered orally once daily for 10 consecutive days beginning on Day 3 after tumor implantation.
†Rats were inoculated iv with 1×10^7 cells of AH 41C ascites hepatoma on Day 0.
‡$P < 0.05$.
§$P < 0.01$.
¶$P < 0.001$.

enhance survival prolongation of rats implanted with AH 41C, the mortality percentage is most effectively reduced by orally administered minimum doses of 200 mg/kg/day. This compound was found to have low toxicity in experimental animals. The LD50 of A-924 was > 1.5 g/kg when given ip and > 4.5 g/kg when given orally in single doses to mice. No teratogenic or adverse effects were observed in normal or pregnant rats and mice when A-924 was given either orally or ip (unpublished observation). Results of our experiments show that A-924 possesses unique antitumor qualities and may be developed as a new type of antitumor drug.

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Phase II Clinical Trial of Ftorafur in 5-Fluorouracil-Refractory Colorectal Cancer [1,2,3]

Brief Reports and Preliminary Communications

Thomas Buroker,* Arnold Miller, Laurence Baker, Michael McKenzie, Michael Samson, and V. K. Vaitkevicius [4]

The pyrimidine nucleoside ftorafur was synthesized in Latvia in 1966 (1). Laboratory studies have shown ftorafur to be active in Sarcoma 180, Walker carcinosarcoma, alveolar liver cancer PC-1, and Harding-Passey melanoma (2). Human clinical trials have demonstrated this 5-fluorouracil (5-FU) analog to have less hematologic toxicity than 5-FU (3,4). Valdivieso et al (4) found doses of 2.0–2.5 g/m²/day × 5 days to be active, with central nervous system (CNS) toxicity the limiting factor. Recently Garibjanian et al (5) have shown cross resistance of ftorafur and 5-FU in L1210 leukemia. Cross resistance in man has not been studied and since the tumor spectrum of a parent compound and its analogs may vary, a phase II trial of ftorafur in 5-FU-refractory colorectal cancer was conducted.

MATERIALS AND METHODS

Twenty-two patients with biopsy-proven disseminated colorectal cancer were entered in the study. All patients had documented progressive disease while receiving 5-FU alone or in combination with mitomycin C or methyl-CCNU. Progression was defined as an increase in the size of measurable lesions by ≥ 50% or the development of new metastatic sites after having received two courses of 5-FU. 5-FU toxicity manifested in all patients as mucositis and/or myelosuppression. Three patients had a partial remission (PR) while receiving a 5-FU combination. All patients had been off their previous chemotherapy for 3 weeks and had definite progressive disease at the time ftorafur therapy was begun. Ten women and 12 men ranging in age from 28 to 78 years were treated. The median performance status was 70 with a range of 60–90. Prior to treatment all patients had a wbc count > 4000/mm³, a platelet count > 100,000/mm³, and a normal renal function. Pretreatment liver scan, chest roentgenogram, and enzymatic liver profile were also obtained. Patients had a life expectancy of ≥ 8 weeks and signed a consent form in accordance with our institutional policy.

Ftorafur was administered as a 2-hour iv infusion at a dose of 2.25 g/m²/day × 5 days and was repeated every 3 weeks. There was no dose escalation in any patient. Blood pressure was monitored every 15 minutes during the infusion and for 2 hours after its completion. Wbc and platelet counts were checked weekly and tumor measurements were recorded every 3 weeks. To be evaluable for toxicity and response, a patient must have survived 3 and 6 weeks respectively.

A PR was defined as a reduction of ≥ 50% of the sum of the products of the longest perpendicular diameters of all measurable lesions. A positive liver scan was required for patients whose liver was to be followed clinically as the measurable site. A remission in these patients was defined as a decrease of ≥ 30% in the sum of the measurements below the xiphoid process and both costal margins at the midclavicular lines. Tumor reduction was required for a minimum of 4 weeks to be accepted as a response.

RESULTS

Eighteen of 22 patients treated with ftorafur were evaluable for toxicity and response. Four patients died with tumor progression within 14 days after beginning therapy. A total of 39 courses were administered to the 18 evaluable patients. Fifteen patients received two courses and three received three courses.

The toxic effects encountered are illustrated in table 1. Predominant side effects consisted of GI intolerance and CNS disturbances. Twenty of the 39 courses were associated with nausea/emesis. One

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[2]Supported in part by contract N01-CM-67105 from the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health, Department of Health, Education, and Welfare.

[3]Ftorafur: NSC-148958; CAS reg. No. 17902-23-7; uracil, 5-fluoro-1-(tetrahydro-2-furyl)-. Supplied by the Developmental Therapeutics Program, DCT, NCI.

[4]Department of Oncology, Wayne State University School of Medicine, Detroit, Mich.

**Reprint requests to:* Dr. Thomas Buroker, Department of Oncology, Wayne State University School of Medicine, 540 E Canfield Ave, Detroit, Mich 48201.

TABLE 1.—Ftorafur toxicity (39 courses)

Toxic effect	No. of courses
GI	
Nausea/emesis	20
CNS	
Lethargy	4
Confusion	2
Hematologic	
Leukopenia (cells/mm³)	
3000–4000	3
2000–3000	1
Thrombocytopenia (cells/mm³)	
75,000–100,000	2
50,000–75,000	1

patient required iv fluids for protracted vomiting. Six of 39 courses resulted in CNS disturbances, primarily lethargy. Lethargy usually began within 1 hour of the ftorafur infusion and persisted through the completion of the fifth day of therapy. Myelosuppression that occurred was mild and tolerable. Orthostatic hypotension was not noted in any patient.

No remissions were noted in any patients. After receiving a minimum of two courses of ftorafur all 18 patients demonstrated increasing metastatic disease.

DISCUSSION

Twenty-two patients with metastatic colorectal cancer, all of whom were refractory to 5-FU, were treated with ftorafur on a daily × 5 schedule. Toxic effects which consisted primarily of nausea, vomiting, and lethargy were tolerable but did not allow for dose escalations. The lack of myelosuppression, the gastrointestinal (GI) intolerance, and the central nervous system (CNS) toxicity encountered in this study were similar to those reported by Smart et al (3) and Valdivieso et al (4).

Eighteen patients, all of whom received a minimum of two courses of ftorafur with at least one course demonstrating toxicity (GI, CNS, or hematologic), showed no response to ftorafur.

Since pharmacologically, ftorafur appears to act as a depot 5-FU with plasma levels which stimulate continuous 5-FU infusions, the resistance of our patients to ftorafur after proven resistance to prior 5-FU is not surprising (6,7).

Although the likelihood of a response is small when a second-line agent is used in patients with colorectal cancer who have failed on 5-FU or a 5-FU combination, we believed it was necessary to document cross-resistance between 5-FU and ftorafur in one specific tumor, since previous studies have not done so. As a result of this study no further trials with ftorafur in patients previously treated with 5-FU are suggested.

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Iv Methyl-CCNU and Ftorafur With or Without Methanol-Extracted Residue of BCG for Metastatic Adenocarcinoma of the Colon [1,2]

Brief Reports and Preliminary Communications

Charles H. Diggs,* Peter H. Wiernik, and A. Collier Smyth [3]

The combination of methyl-CCNU and 5-fluorouracil (5-FU) with or without vincristine has shown higher response rates in adenocarcinoma of the colon than have been reported with single agents or with other combinations (1–5). In order to study a variant of this combination, the Baltimore Cancer Research Center initiated a chemotherapeutic study of metastatic adenocarcinoma of the colon and rectum using the combination of a nitrosourea and a 5-FU-like drug, with or without immunotherapy using the methanol-extracted residue (MER) of BCG. The iv form of methyl-CCNU was chosen in an attempt to decrease the nausea and vomiting that is associated with the oral form of this agent. Phase I studies of iv methyl-CCNU have confirmed the feasibility of its administration, and dosages approximating 75% of the equally toxic oral form have been suggested for further trials (6). Ftorafur, a drug first used in the Soviet Union, was chosen for use since early reports have suggested that it has activities equal to that of 5-FU but is associated with less myelosuppression and mucosal toxicity (7). Immunotherapy with MER was studied after initial reports from the Mayo Clinic suggested that MER as a single agent had activity in this disease (8).

PATIENTS AND METHODS

Twenty-one patients (median age, 56 years) who had metastatic adenocarcinoma of the colon or rectum with lesions that could be objectively evaluated were treated with the present combination. Eighteen of the 21 patients treated were fully ambulatory at the start of treatment. One patient had received prior chemotherapy and four had received local radiation therapy for their disease.

Methyl-CCNU, 130 mg/m^2 iv, Day 1

+

Ftorafur, 2 g/m^2 iv, Days 1-5, 21-25

±

MER, 1 mg intradermally, Days 1, 12, 22

FIGURE 1.—Chemotherapy for metastatic colon adenocarcinoma.

Thirteen patients had hepatic involvement with metastatic disease either alone or in combination with other metastatic sites.

All patients were treated with the regimen as shown in figure 1. Methyl-CCNU was prepared in a 10% fat emulsion with 5% ethanol and was given by means of a Harvard pump as previously described (9). Ftorafur was prepared by initially reconstituting the contents of the NCI-prepared vial with sterile water for injection, followed by redilution of the total dose to 150 ml using 5% dextrose in water. Each dose was given by slow iv drip infusion over 45–60 minutes. In addition, 13 patients were randomized to receive MER. Doses of methyl-CCNU were adjusted in successive courses according to the hematologic toxicity experienced during the previous course. Ftorafur doses on Day 21 were not attenuated unless the wbc count was $< 2000/mm^3$ or the platelet count was $< 50,000/mm^3$. Doses were withheld until these levels were achieved. Courses were repeated every 6 weeks.

Partial remission (PR) was defined as a $> 50\%$ reduction in the product of the perpendicular measurements of all sites of measurable disease without evidence of tumor progression at new sites. Stabilization was defined as either $< 50\%$ reduction in size of the lesions or no increase in their size for at least 6 weeks.

RESULTS

No patient treated experienced total regression of measurable disease. Two patients treated without MER were able to achieve a PR and four patients, three of whom had received MER, achieved stabilization of disease. One patient who achieved a PR

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[2]Ftorafur: NSC-148958; CAS reg. No. 17902-23-7; uracil, 5-fluoro-1-(tetrahydro-2-furyl)-.

[3]Baltimore Cancer Research Center (BCRC), Division of Cancer Treatment, National Cancer Institute (NCI), Md.

*Reprint requests to: Charles H. Diggs, MD, BCRC, University of Maryland Hospital, 22 S Greene St, Baltimore, Md 21201.

had an abdominal mass that was considered surgically nonresectable after two previous surgical procedures. This PR lasted 31 weeks before progression in the size of the mass was noted. The other patient who achieved a PR had a left neck mass that is presently still responsive at 9+ weeks. Stabilization of disease lasted 4, 8, 13, and 14 weeks. The other 15 patients treated progressed while receiving chemotherapy. Patients with responding or stable disease received a median of three courses of therapy; nonresponders received a median of one and one half courses. Median survival for all patients was 17 weeks.

Severe myelosuppression was the primary toxic effect in this regimen (table 1). Iv methyl-CCNU administration was associated with mild nausea and very little vomiting. Ftorafur administration was associated with a feeling of light-headedness in several patients, although this was not common and could usually be controlled by slowing the infusion rate of the medicine. Neither mucosal toxicity nor diarrhea was associated with ftorafur administration. Vomiting occurred, but was moderate in intensity. MER administration was associated with fevers as high as 104°F, and tender, erythematous, raised lesions at each site of its administration. It was not uncommon for these lesions to exhibit reactivation when further doses of MER were given.

TABLE 1.—Chemotherapy toxicities (21 patients)

	No. of patients
Platelet count/mm³	
< 100,000	13 (62%)
< 50,000	10 (48%)
Wbc count/mm³	
< 2000	7 (33%)

DISCUSSION

Although the combination of oral methyl-CCNU and 5-FU has been shown to be beneficial in advanced metastatic colorectal adenocarcinoma, we have been unable to achieve a similar response rate with similar though not identical drugs. There could be several possible explanations for this lack of efficacy.

First, iv methyl-CCNU has unknown activity in colorectal carcinoma. The oral preparation does have an 11%–25% response rate for this disease (10,11). Since the hematologic toxicity seen here was compatible with that seen with the nitrosoureas, the iv method of drug administration does possess biologic activity, but may not have antitumor effect.

Second, when stricter criteria are applied the actual PR rate in the Soviet studies with ftorafur was 7% rather than the 44% "response" reported (7). These results would seem inferior to the PR rates seen with 5-FU of 8%–25% (12–14), but further studies comparing the two drugs are needed.

Also, it may be that ftorafur requires activation to 5-FU to have antineoplastic properties. Since animal studies have suggested that this metabolic conversion occurs in hepatic tissue and since many of the patients treated here had hepatic dysfunction, it is possible that no activation of ftorafur occurred in our patients. Neither of the two patients who achieved PR had obvious hepatic involvement, lending support to this theory.

Immunotherapy as given here likewise did not cause any increase in the response rate, although Moertel et al (8) have reported three responses to MER alone. The toxicity of MER was only marginally acceptable to our patients since its administration was associated with fever, pain, and open sores without therapeutic benefit. Indeed, if one compares the 25% PR rate (two of eight patients) of those who received no MER with the 0 response rate (none of 13 patients) of MER-treated patients, this form of immunotherapy would seem to be detrimental.

In summary, this treatment regimen was not as effective as previously reported similar combinations in carcinoma of the colon and rectum. Possible reasons for this lack of efficacy have been reviewed. Further studies with ftorafur, especially of its metabolic pathway in man, are needed to determine whether any activation of the drug occurs in patients with liver disease and whether or not it offers any advantages over 5-FU.

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Single-Agent Therapy for Renal Cell Carcinoma: CCNU, Vinblastine, ThioTEPA, or Bleomycin [1]

Brief Reports and Preliminary Communications

Davis M. Hahn, Stephen C. Schimpff,* John C. Ruckdeschel, and Peter H. Wiernik [2]

Metastatic renal cell carcinoma has proven to be generally unresponsive to all agents evaluated to date including most alkylating agents (1–3), antimetabolites (2), and antibiotics (3). Progesterone derivatives or androgens give a low number of partial remissions (PRs) and about a 20% subjective response rate (1,4–6). Many currently available chemotherapeutic agents have not yet been adequately evaluated (2) including three agents for which there are preliminary data to suggest some responsiveness, ie, CCNU (2,7,8), vinblastine (1–3,9), and thioTEPA.[3] These three drugs were therefore tested in a prospective, randomized trial for therapeutic efficacy in patients with metastatic renal cell carcinoma. Patients whose tumor progressed on this study were then treated with bleomycin for which there are also some early data suggesting possible value in this disease (10).

METHODS AND MATERIALS

Patient population.—Patients with histologically documented metastatic renal cell carcinoma who were admitted to the BCRC between February 1974 and June 1976 were randomized to receive one of three drug regimens. An informed consent was required prior to therapy. Patients were excluded only if they had had previous cytotoxic chemotherapy.

Documentation of extent of disease.—All patients had (*a*) complete history and physical examination, (*b*) chest roentgenogram and whole lung tomography, (*c*) technetium 99 polyphosphate liver-spleen scan, (*d*) the following serum studies: urea nitrogen, creatinine, electrolytes, and SMA-12, and (*e*) complete blood cell count with leukocyte differential. Technetium 99 polyphosphate bone scan or radiographic bone survey, bone marrow biopsy, whole body gallium 67 citrate scan, and technetium 99 polyphosphate brain scan were performed when clinically indicated.

Drug dose, scheduling, administration, and modification.—Patients were randomized by sealed cards to receive one of the three following agents.

A.—CCNU (Bristol Laboratories), 130 mg/m^2, was given orally every 6 weeks. One-half dose was given if the wbc count nadir was $<2500/mm^3$ or the platelet count nadir was $<100,000/mm^3$. Repeat doses were withheld until the wbc count returned to $>3500/mm^3$ and the platelet count to $>150,000/mm^3$.

B.—Vinblastine (Eli Lilly Co), 0.15 mg/kg, was given iv every week. One-half dose was given if the wbc count nadir was between 2500 and $3500/mm^3$ or if the platelet count nadir was between 100,000 and $150,000/mm^3$, or if constipation or paresthesias developed. Repeat dosage was withheld until the wbc count was $>2500/mm^3$, the platelet count was $>100,000/mm^3$, and severe constipation or severe neurologic sequelae had abated.

C.—ThioTEPA (Lederle Laboratories), 20 mg/m^2, was given iv every week. Dose modification for myelosuppression was the same as that used for vinblastine.

Subsequent therapy.—Patients who failed to respond to initial therapy and who had normal pulmonary function were then given bleomycin, 15 mg/m^2, im every week with monthly pulmonary function studies.

Evaluation of response.—Standard response categories were employed: complete remission (CR)—disappearance of all demonstrable lesions and normalization of all abnormal laboratory values; PR—$>50\%$ decrease in the sum of the products of diameters of all measurable lesions; objective response—a tumor shrinkage $<50\%$; stabilization—tumor size unchanged during at least 2 months of therapy; and progression—$>25\%$ increase in the sum of the products of diameters of any measurable lesions.

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[2]Baltimore Cancer Research Center (BCRC), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Md.

[3]Holland JF. Personal communication.

**Reprint requests to:* Stephen C. Schimpff, MD, BCRC, DCT, NCI, University of Maryland Hospital, 22 S Greene St, Baltimore, Md 21201.

TABLE 1.—Chemotherapy results

	Treatment			
	Primary			Secondary
	CCNU	Vinblastine	ThioTEPA	Bleomycin
No. of patients entered	9	10	7	7
No. of patients evaluable	7	10	7	7
Mean No. of courses of therapy (range)	2(1–3)	4.7(1–12)	8.8(1–27)	7.6(5–12)
No. of responses				
CR	0	0	0	0
PR	0	0	1	0
Objective	0	2	0	0
Stabilization	1	1	1	2
Progression	6	7	5	5

RESULTS

Patient Population

Twenty-six patients were randomized; ten received vinblastine, nine received CCNU, and seven received thioTEPA. There were 16 men and ten women. The mean age was 52 years. The median time from diagnosis to therapy was 7 months. Twenty-four patients had pulmonary metastases; three of eight patients tested because of clinical suspicion had abnormal brain scans and/or skull films. Twelve of 15 patients had abnormal bone scans or bone surveys. Abdominal mass was present by palpation in six patients, and three had abnormal iv pyelograms or arteriograms which, in addition to the metastatic disease, could be followed in order to determine a response.

CCNU.—Seven of nine patients were evaluable, one being lost to followup after one course and one refusing further therapy (table 1). Six patients showed clear progression while one patient, still alive 2 years later, has had stable disease. Four of seven patients had myelosuppression sufficient to require dose reduction. Only one patient had a wbc count nadir $< 2000/mm^3$ and three patients had a platelet count nadir $\leq 40{,}000/mm^3$. Only two of the four patients who had myelosuppression had had previous radiotherapy. Vomiting occurred in four patients.

Vinblastine.—Two of ten patients treated with vinblastine had an objective response (table 1). One of these had disappearance of an abdominal mass while his pulmonary metastases remained unchanged. The other had a $> 50\%$ reduction in her lung lesion but no change in a lytic pelvic lesion. Both responses were for 2-month periods. Additionally, one patient had stabilization in pulmonary nodules during 20 courses of vinblastine. Side effects included mild neurotoxicity in two patients after three and 27 courses, mild constipation in two patients after nine and 27 courses, mild leukopenia in three patients, and severe leukopenia with a wbc count $< 1000/mm^3$ for 1 day in one patient. Thrombocytopenia did not occur. Drug dose was decreased in four patients because of myelosuppression.

ThioTEPA.—Seven patients received thioTEPA (table 1). One patient had a PR of her pulmonary metastases for 1 month. Myelosuppression, which occurred in three patients, was the only side effect. The wbc count nadirs were $2900/mm^3$, $2200/mm^3$, and $1200/mm^3$ while the platelet count nadirs were $92{,}000/mm^3$, $35{,}000/mm^3$, and $9000/mm^3$. Only one of these patients had received previous radiotherapy. The three patients who had myelosuppression had to have dose reduction and one patient had to have therapy withheld for 1 month because of prolonged myelosuppression.

Bleomycin.—Because of death or pulmonary insufficiency, only seven patients received bleomycin; no responses occurred (table 1).

Survival

The median times from diagnosis to death and treatment to death for vinblastine (10 and 5 months), CCNU (15 and 10 months), and thioTEPA (16 and 7 months) are not significantly different from each other or from untreated patients with metastatic hypernephroma (9).

DISCUSSION

The chemotherapeutic agents used in this study failed to show any substantial activity against renal cell carcinoma or any change in survival results in

a preliminary study of a small number of previously untreated patients. ThioTEPA and vinblastine demonstrated minimal activity (one PR and two objective responses, respectively). Although other studies (7,9) have reported a higher response rate for CCNU or bleomycin, no responses were seen in those patients treated with CCNU or bleomycin.

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5-Fluorouracil Versus CCNU in the Treatment of Metastatic Prostatic Cancer [1,2]

Brief Reports and Preliminary Communications

Francisco Tejada,[3,*] Mario A. Eisenberger,[3] Lawrence A. Broder,[3] Martin H. Cohen,[4] and Richard Simon [5]

From September 1973 to May 1975, a randomized study was carried out to compare the effect of 5-fluorouracil (5-FU) versus CCNU in the treatment of patients with symptomatic metastatic prostatic cancer who failed to respond to hormonal manipulation.

MATERIAL AND METHODS

Sixteen patients with histologically proven metastatic adenocarcinoma of the prostate were randomly allocated into 19 therapeutic trials. All patients were hormonal therapy failures and were symptomatic with bone pain and/or significant (> 10%) weight loss in the 4 weeks prior to entering the study.

Patients were evaluated before entering the study and then serially every 4 weeks for weight, performance status (Zubrod scale) (1), measurement of the area (cm^2) of metastatic lesions, complete blood cell counts, liver and renal function tests, and peripheral blood and bone marrow acid phosphatase (colorimetric assay, Phosphatabs-Acid Quantitative Method, Warner-Chilcott, Morris Plains, NJ; normal values = 0.5–5.0 Babson-Read units) (2).

Patients were stratified for extent of disease and acid phosphatase level. The treatment schema is outlined in figure 1.

Response to therapy was evaluated as objective or subjective. Objective response (complete or partial) was the improvement in two or more of the following parameters: a > 50% decrease in the size of one or more measurable lesions in the absence of progressive disease; a > 50% decrease in peripheral blood and/or bone marrow acid phosphatase; an improvement in performance status by two categories or a weight gain of > 10% in a 4-week period not due to edema. Subjective response was defined as the improvement in performance status by two categories in the absence of progressive disease.

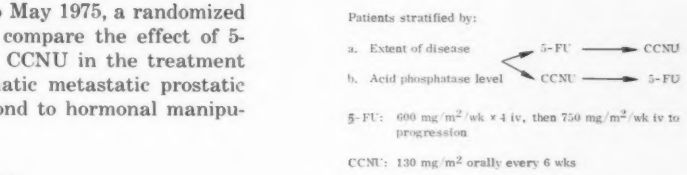
Patients stratified by:
a. Extent of disease
b. Acid phosphatase level
5-FU → CCNU
CCNU → 5-FU
5-FU: 600 mg/m²/wk × 4 iv, then 750 mg/m²/wk iv to progression
CCNU: 130 mg/m² orally every 6 wks

FIGURE 1.—Initial chemotherapeutic trials in patients with inoperable or recurrent cancer of the prostate (protocol MOB-73-02, NCI-VA Medical Oncology Branch, Sept 1973).

No response to therapy was evaluated as progression or no change of disease. Progression was a ≥ 50% increase in measurable disease (or the appearance of new lesions) or a ≥ 50% increase in serum or bone marrow acid phosphatase. Decrease in performance status by two or more levels or a >10% weight loss within 4 weeks also constituted progressive disease. No change of disease referred to those patients in whom neither response or progression could be documented. Toxicity was evaluated as described in a previous report (2).

RESULTS

Eighteen of the 19 treatment trials in the study were considered evaluable. One patient died of progressive disease within 4 weeks of treatment with 5-FU and was considered unevaluable for response. The results of evaluable trials are shown in table 1.

The proportion of patients without disease progression at different times of study in the 5-FU versus CCNU groups was different for the patients considered to have a partial response, with a median duration of response of 100 days for 5-FU and 186 days for CCNU.

Table 2 indicates the toxicity and how it was evaluated.

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[2]This work was conducted at the National Cancer Institute (NCI)-Veterans' Administration (VA) Medical Oncology Branch, Washington, DC, and drugs were supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment (DCT), NCI, Bethesda, Md.

[3]Department of Oncology and Comprehensive Cancer Center for the State of Florida, University of Miami School of Medicine.

[4]NCI-VA Medical Oncology Branch, DCT, NCI, Washington, DC.

[5]DCT, NCI, Bethesda, Md.

**Reprint requests to:* Francisco Tejada, MD, University of Miami, PO Box 520875, Biscayne Annex, Miami, Fla 33136.

TABLE 1.—Treatment response

Treatment	No. of evaluable trials	Response (No. of patients)				% partial response
		Partial	Symptomatic	No change	Progression	
5-FU	8	2	0	1	5	25
CCNU	10	4	0	2	4	40

TABLE 2.—Toxic effects of therapy

Treatment	No. of patients	No. of patients with toxic effects*				
		Infection	Wbc count	Platelet count	Heart failure	Nausea and vomiting
5-FU	8	1 (4+)	3 (1–4+)	6 (1–3+)	0	4 (1–2+)
CCNU	10	1 (3+)	3 (1–3+)	4 (1–3+)	0	4 (1–2+)

*Nos. in parentheses are degrees of toxicity:

Degree	Infection and nausea and vomiting	Wbcs/mm³	Platelets/mm³
1+	Slight	< 4000	< 150,000
2+	Moderate	< 3000	< 100,000
3+	Marked	< 2000	< 75,000
4+	Life-threatening	< 1000	< 50,000

DISCUSSION

Cytotoxic chemotherapy studies in patients with prostatic cancer have indicated that cyclophosphamide produces a 31% response rate (3), 5-FU yields rates of 29% (4) and 36% (5), and adriamycin is associated with a partial response rate of 26% (6). This report supports the finding that 5-FU has modest activity; our study produced a partial response rate of 25% and the responses have been short in duration. Interestingly, treatment with a nitrosourea, CCNU, produced partial responses in 40% of the patients (table 2). The responses to CCNU have been sustained for periods of from 6 to 10 months.

Toxicity from 5-FU and CCNU therapy at the doses and schedules used has been moderate but was transient and tolerable by the patients.

This study indicates that CCNU may potentially be one of the more active agents in the treatment of patients with metastatic prostatic cancer who have failed to respond to primary hormonal therapy.

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CCNU, Bleomycin, and Methylprednisolone With or Without Adriamycin in Renal Cell Carcinoma: A Randomized Trial [1,2]

Brief Reports and Preliminary Communications

Frederick Richards II,* Hyman B. Muss, Douglas R. White, M. Robert Cooper, and Charles L. Spurr [3,4]

Metastatic renal cell carcinoma is associated with a very poor prognosis, and most patients with this disease survive < 2 years. Chemotherapy has generally been disappointing, with very few studies showing a > 20% response rate (1–3). Because of initial encouraging results with CCNU (4,5) as well as bleomycin (6), the present study using both agents was undertaken. At the time of initiation of this study, only preliminary evidence concerning the efficacy of adriamycin in renal cell carcinoma was available, and because of encouraging results in other adenocarcinomas, adriamycin was added to one of our chemotherapy arms.

MATERIALS AND METHODS

Thirty patients with histologically confirmed renal cell carcinoma, seen between July 1974 and December 1976, were entered into this study. Only patients with measurable disease were eligible for study. Patients were excluded from the study if they had had prior exposure to CCNU, bleomycin, or adriamycin, if the wbc count was < 4000/mm³ or the platelet count < 100,000/mm³, or if the BUN was > 25 mg/100 ml or the creatinine > 1.5 mg/100 ml. No patient had received chemotherapy or irradiation therapy for at least 2 weeks prior to entry into this study. Written informed consent meeting all institutional, state, and federal guidelines was obtained from all patients. Prior to entry into the study, the following data were obtained on all patients: history and physical examination, cbc, SMA-C, platelet count, chest roentgenogram, skeletal survey, bone scan, brain scan, liver scan, and electrocardiogram. Indicator lesions were measured and recorded.

After entry into the study, all patients were randomly assigned to one of two treatment regimens. Regimen I consisted of CCNU and bleomycin and regimen II consisted of CCNU, bleomycin, and adriamycin. CCNU was given orally every 6 weeks at a dose of 100 mg/m²; a cycle of therapy was defined as one course of CCNU. The bleomycin dose was 7.5 mg/m² iv given twice weekly for 3 weeks, then once weekly for 6 weeks, and then once every 2 weeks to a final cumulative dose of 300 mg. In order to minimize febrile reactions, each bleomycin infusion was immediately followed by 40 mg iv of methylprednisolone sodium succinate. Methylprednisolone probably should not have been used in this drug trial because it was given at high enough doses that it might be considered chemotherapy. In regimen II, adriamycin, 30 mg/m², was given iv every 3 weeks commencing on Day 1 with CCNU and continued to a final cumulative dose of 540 mg/m². Cbc and platelet counts were obtained weekly during the first 9 weeks of therapy and subsequently prior to each course of adriamycin or CCNU. Adriamycin and CCNU were only given when the wbc count was > 4000/mm³ and the platelet count was > 100,000/mm³. Bleomycin was omitted only for a wbc count < 2000/mm³ or a platelet count < 50,000/mm³.

Response to therapy was assessed at 6-week intervals. A complete response (CR) indicated the complete disappearance of all measurable disease. Partial response (PR) was defined as a 50% reduction of the product of the longest perpendicular diameters of the indicator lesion(s) with no new lesions observed. When malignant hepatomegaly was chosen as the indicator, at least a 50% decrease in the sum of the measurements below the right and left costal margins in the midclavicular line

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[3] Oncology Research Center, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC.

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* *Reprint requests to:* Frederick Richards II, MD, Department of Hematology/Oncology, The Bowman Gray School of Medicine, Winston-Salem, NC 27103.

TABLE 1.—Pretreatment data and patient response

	Regimen I (16 patients)	Regimen II (14 patients)	Total (30 patients)
Median age (men/women)	60/59	56/61	58/60
No. of men (%)	8(50)	9(64)	17(57)
No. of patients (%) with—			
Disease-free interval of 18 mos	15(94)	12(86)	27(90)
Prior treatment			
Removal of primary tumor	15(94)	11(79)	26(87)
Chemotherapy	1(6)	1(7)	2(6)
Disease site			
Renal mass	2(13)	5(36)	7(23)
Lung	10(63)	7(50)	17(57)
Bone	8(50)	3(21)	11(37)
Liver	2(13)	4(29)	6(20)
Other	7(44)	6(43)	13(43)
Responses			
CR	1(6)	0	1(3)
PR	0	3(21)	3(10)
Stable	4(25)	5(36)	9(30)
Progression	11(69)	6(43)	17(57)
Duration (mos)	4*	3, 3, 18	
No. of treatment cycles per patient			
Median	2	2	2
Range	1–7	1–6	1–7
No. of patients given 1 cycle only	5(31)	5(29)	9(30)
Median time (wks) in study	12.7	12.0	12.4
Median survival (wks)	16.6	17.7	17.5

*Died in CR.

with stabilization or improvement of liver function tests was required for PR. All objective responses whether complete or partial had to be accompanied by significant subjective improvement and had to persist for at least 6 weeks. Progressive disease was defined as the appearance of new lesions or a $> 50\%$ enlargement in an indicator lesion as measured above. Patients whose disease did not qualify for any of the above categories were considered to have stable disease.

RESULTS

Sixteen patients were assigned to regimen I and 14 to regimen II. Patient characteristics and response to therapy are noted in table 1. The single CR in regimen I occurred in a patient who had biopsy-confirmed left supraclavicular lymph node metastases as the only evidence of metastatic disease. This patient had complete resolution of supraclavicular lymphadenopathy and died in CR 4 months after therapy because of cardiovascular disease. The three PRs in regimen II included a patient with pulmonary metastases only who had a PR for 3 months. The second patient, with a large flank mass, also had a PR for 3 months. A third patient, with biopsy-proven hepatic metastasis, achieved a PR for 18 months. In this patient, dramatic improvement occurred in both the liver scan and the physical examination. This patient has subsequently relapsed. Progressive disease was noted in more than half of the patients and seemed unaffected by chemotherapy. There was no significant difference in response rates between the two groups. Secondary therapy with progestins or other chemotherapeutic agents was given to ten patients with progressive disease. All of these patients were given a progestational agent, and six were given vinblastine in addition. One patient with subcutaneous metastases as the only evidence of recurrent disease had a CR to im progesterone which lasted 6 months. There were no other responders to hormonal therapy or chemotherapy in this group. There was no significant difference between the two regimens and, overall, both patient groups did very poorly.

TOXICITY

Hematologic toxicity was acceptable in both programs and no patient died from infection or bleeding complications related to therapy. Leukopenia was more pronounced in patients receiving adriamycin. One patient died of apparent bleomycin pulmonary toxicity. This patient with a large recurrent flank mass had an objective (PR) response to therapy. Death was due to pulmonary insufficiency, secondary to progressive pulmonary infiltrates, with no evidence of bacteriologic or other infectious etiology. Unfortunately, a post-mortem examination could not be obtained. Because of the brief duration of responses, few patients received high total doses of bleomycin. Fever and chills were rarely seen after bleomycin due to the concurrent administration of corticosteroids. Vomiting due to CCNU was extremely common. Alopecia occurred in all patients receiving adriamycin.

DISCUSSION

The combination of CCNU, bleomycin, and methylprednisolone, with or without adriamycin, does not appear to offer any therapeutic advantages over other regimens for the treatment of renal cell carcinoma. While many of the patients were seriously ill upon entry into the study, the failure to obtain a substantial response to therapy was due to tumor resistance rather than host debilitation. Single-agent chemotherapy continues to be disappointing in this disease, and phase I and II trials with new agents as well as newer combination approaches are needed.

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Adriamycin and 5-Fluorouracil in the Treatment of Advanced Hepatoma: A Southwest Oncology Group Study [1,2]

Brief Reports and Preliminary Communications

Laurence H. Baker, John H. Saiki, Stephen E. Jones, James S. Hewlett, Robert W. Brownlee, Ronald L. Stephens, and Vainutis K. Vaitkevicius [3,*]

Renewed interest in the medical treatment of hepatoma has recently occurred as a result of the experience reported by Olweny et al (1) using adriamycin in African patients with advanced hepatoma. Almost simultaneously, three cooperative groups (Eastern Cooperative Oncology Group [ECOG], Southeastern Cancer Study Group [SECSG], and SWOG) (2–4) and other investigators (5,6) have initiated studies using adriamycin.

In the past, occasional benefit to hepatoma patients treated with 5-fluorouracil (5-FU) has been reported (7). Bard et al (8) at Albany reported on the usefulness of a single-dose intermittent schedule of 5-FU given every 3 weeks. Since the most commonly employed schedule of adriamycin is also every 3 weeks, the combination of 5-FU and adriamycin given every 3 weeks appeared to be a schedule of maximum convenience to the patient and the clinic. Previously, a broad phase I–II trial was conducted at Wayne State University (9) utilizing 5-FU at 500 mg/m² every 3 weeks and adriamycin at 50 mg/m² every 3 weeks. From this trial we learned that this combination produced reproducible, predictable, and manageable toxicity. Two patients from that trial with hepatoma achieved clinical remission.

This paper presents the results obtained by the SWOG using a combination of adriamycin and 5-FU in the treatment of advanced hepatoma in 38 American patients.

MATERIALS AND METHODS

Thirty-eight patients from 18 institutions were entered into this clinical trial. There were 28 men and ten women with a median age of 56 years (range, 19–74 years). All patients had unresectable hepatocellular carcinoma confirmed by surgery or clinical evaluation and histologically confirmed by the institutional pathologist. Median initial chemistry determinations of SGOT, LDH, alkaline phosphatase, and serum bilirubin were 100, 250, and 195 units, and 1.2 mg/100 ml, respectively. All patients had disease that was measurable. No patient had received prior chemotherapy. Initial evaluation included history and physical examination, cbc and platelet counts, liver and renal function studies, and appropriate radiographic and isotopic scanning procedures. Patients were categorized as good- or poor-risk based upon the presence or absence of hyperbilirubinemia (bilirubin ≥ 2.0 mg/100 ml). Good-risk patients received 50–60 mg/m² of adriamycin and 500–600 mg/m² of 5-FU iv every 3 weeks. Poor-risk patients (elevated serum bilirubin levels) received 30–40 mg/m² of adriamycin and 500–600 mg/m² of 5-FU iv every 3 weeks. There were 21 patients categorized as good-risk (median performance status = 80) and 17 as poor-risk (median performance status = 60). A response was defined as $\geq 50\%$ objective decrease in the product of the longest perpendicular diameters of the tumor. Additional requirements of a partial response included stabilization or improvement in performance status and a response lasting for > 1 month. If hepatomegaly was the sole indicator of disease, it was required that the sum of measurements below the xiphoid and subcostal margins decrease by at least 33%. Although initial and serial determinations of serum alpha-fetoprotein (AFP) were recommended these were not used as criteria of response. Therefore, no

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[3]This study was performed by the Southwest Oncology Group (SWOG). Participants in the study were: *L. H. Baker* (Wayne State University Medical School, Detroit, Mich); *J. H. Saiki* (University of New Mexico School of Medicine, Albuquerque); *S. E. Jones* (University of Arizona School of Medicine, Tucson); *J. S. Hewlett* (Cleveland Clinic, Ohio); *R. W. Brownlee* (Henry Ford Hospital, Detroit, Mich); *R. L. Stephens* (University of Kansas Medical Center, Kansas City); and *V. K. Vaitkevicius* (Wayne State University School of Medicine, Detroit, Mich).

**Reprint requests to:* SWOG Operations Office, 3500 Rainbow Blvd, Suite 100, Kansas City, Kan 66103.

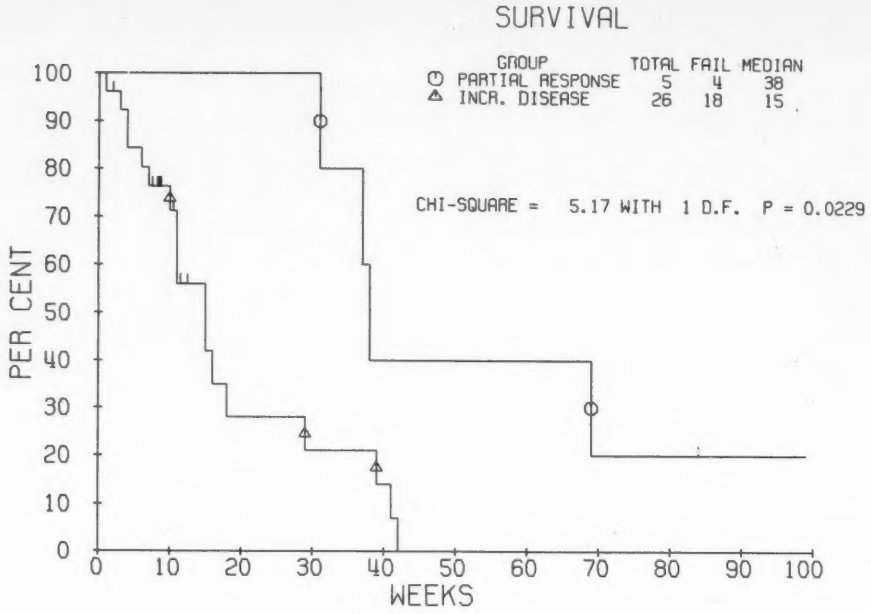

FIGURE 1.—Median survival time for responders and nonresponders.

definitive statement could be made regarding the correlation of AFP levels and response.

RESULTS

A total of 38 patients were eligible for this protocol. Five patients died within 12 days of starting therapy (one good-risk and four poor-risk patients). None of the five patients experienced antitumor or toxic effects from this therapy. Another five patients experienced objective remission (three good-risk and two poor-risk patients). Median performance status of the responders was 80 versus 70 for the nonresponders. All responses occurred in patients whose disease was limited to the liver. Median duration of response was 10 weeks (range, 4–36 weeks). The median survival time for the 26 nonresponding patients was 15 weeks versus a median survival time of 38 weeks for the responders ($P = < 0.02$) (*see* fig 1). Median survival time for the good-risk patients was 34 weeks versus 12 weeks for the poor-risk group ($P = < 0.03$).

Toxicity

The major toxic effects encountered were mild nausea, alopecia, and myelosuppression. Median wbc and platelet nadirs were $3.8 \times 10^3/mm^3$ and $180 \times 10^3/mm^3$, respectively. This schedule was well tolerated by patients and easily suitable for outpatient administration.

DISCUSSION

Thirteen percent of all patients entered experienced remission. Although the median duration of objective response was a relatively brief 10 weeks, survival of the responding patients was significantly longer compared to that observed for the nonresponders. This observation may in part be due to the strict criteria of response or the difficulty in measuring response in this disease.

Our data are quite similar to the data reported by Falkson (ECOG), Vogel (SECSG), and Kane (NCI-Veterans' Administration Medical Oncology Branch) where adriamycin was used as a single agent. This raises doubt as to the value of adding 5-FU in this route and schedule. We did note a significant difference in survival of responders versus nonresponders.

Since only 42% of the patients experienced myelosuppression it may be inferred that insufficient doses were utilized. However, our analysis revealed that patients who demonstrated myelosuppression

did not achieve clinical remission more often than patients who did not have toxic reactions (two of the five responders never experienced myelosuppression). Additionally, survival of the patients experiencing toxic effects was slightly less than survival of those patients who did not. The schedule was well tolerated and, unlike most combination therapies, was relatively simple to administer.

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Vinblastine, 5-Azacytidine, and VP-16-213 Therapy for Previously Treated Patients With Acute Nonlymphocytic Leukemia [1]

Brief Reports and Preliminary Communications

David A. Van Echo,* Karen M. Lichtenfeld, and Peter H. Wiernik [2]

Although there have been significant advances in the primary induction therapy for acute nonlymphocytic leukemia (ANLL) using daunorubicin and cytosine arabinoside together and in various other combinations, this disease invariably becomes resistant to these agents even after prolonged remissions.

Vinblastine, a vinca alkaloid similar to vincristine in activity, is a commercially available antitumor agent that is moderately myelosuppressive and occasionally neurotoxic. It has been shown to have activity in children with monocytic leukemia (1). 5-Azacytidine is a pyrimidine nucleotide analog structurally similar to cytidine, is incorporated into nucleic acids, and affects both RNA and DNA synthesis. It has well-established antileukemic activity (2). Previously, at our institution, a 28% complete remission (CR) rate was obtained in 18 previously treated adult patients with ANLL at a dosage of 200 mg/m²/day × 5 (3). VP-16-213 is a semi-synthetic podophyllotoxin derivative related chemically to VM-26 which is effective in Hodgkin's and non-Hodgkin's lymphoma (4). The podophyllotoxins are cytostatic in vitro, preventing cells from entering mitosis or destroying them in the premitotic phase. VP-16-213 is more active in acute leukemia than VM-26, and its most striking activity appears to be in acute monocytic and myelomonocytic leukemia (5).

Because of the proven activity of these three agents and the lack of cross-reactivity with other agents employed in the earlier treatment of ANLL, this study was undertaken to determine if the combination could improve on the results obtained with 5-azacytidine alone just prior to this study at our institution.

METHODS

Twenty-seven patients with previously treated ANLL were included in this study. There was no randomization and all patients were evaluable. All patients had received intensive prior therapy with daunorubicin, cytosine arabinoside, and other drugs. Six patients had never achieved a remission and the other 21 patients had relapsed from their remissions and become resistant to their initial therapies. The first 15 patients received the combination of vinblastine, 5-azacytidine, and VP-16-213 (VAV). In this group, two patients had previously been treated with 5-azacytidine in combination with methyl-GAG and had had no response (6). The next 12 patients were treated with higher doses of 5-azacytidine and VP-16-213 alone (AV). In addition, two patients who had previously been treated with VAV were subsequently treated with AV to bring the total to 14 patients in the AV group.

In VAV patients, reinduction was attempted with an initial dose of vinblastine, 6 mg/m², injected iv on Days 1 and 5; 5-azacytidine, 150 mg/m²/day, injected iv over 10–15 minutes in three divided doses × 5 days; and VP-16-213, 50 mg/m²/day, given by iv infusion over 1 hour × 5 days. For AV patients the vinblastine was omitted, the initial dose of 5-azacytidine was given as above for 5 days, and the VP-16-213 dose was 75 mg/m²/day by iv infusion over 1 hour × 5 days.

During induction, all patients were hospitalized and examined daily. Complete hemograms were obtained three times a week; liver, renal, and other serum biochemical tests were performed weekly and as the clinical course indicated. Bone marrow aspirations were performed on all patients prior to entry in the study and at weekly intervals after each course of therapy.

The bone marrow response determined subsequent therapy. Doses of 5-azacytidine were increased by 50 mg/m²/day if the bone marrow remained hypercellular with increased numbers of leukemic cells. Courses of therapy were continued until patients achieved remission, developed unacceptable toxicity, or progressed. CR and partial

[1]Received May 12, 1977; revised June 29, 1977; accepted July 20, 1977.

[2]Baltimore Cancer Research Center (BCRC), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Md.

Reprint requests to: David A. Van Echo, MD, BCRC, DCT, NCI, University of Maryland Hospital, 22 S Greene St, Baltimore, Md 21201.

TABLE 1.—Patient characteristics

	VAV	AV
No. of patients	15	14
Men	5	11
Women	10	3
Median age in yrs (range)	37 (17–59)	29 (17–68)
Diagnoses (No. of patients)*		
AML	4	5
AMML	9	7
AMOL	1	1
APL	1	1
AUL	—	1
Median No. of previous remissions† (range)	1.4 (0–3)	1.1 (0–5)
Median duration of survival from diagnosis to entry in study in wks (range)	65 (19–212)	41 (3–113)
Median duration of previous remission in wks (range)	33 (4–111)	24 (5–36)
No. of patients not previously in remission	2	4‡
Median pretreatment wbc/mm³ (range)	6000 (800–17,000)	14,800 (300–76,000)

*AML = acute myeloblastic leukemia; AMML = acute myelomonocytic leukemia; AMOL = acute monoblastic leukemia; APL = acute progranulocytic leukemia; AUL = acute undifferentiated leukemia.

†Refers to both CRs and PRs.

‡Includes 1 patient who had no response to 3 courses of VAV.

remission (PR) were diagnosed according to previously published criteria (7). After patients achieved a CR they were followed at monthly intervals until they relapsed in the bone marrow. Reinduction was then attempted with the prior successful regimen.

Vigorous and full supportive care was given to all patients including prophylactic oral non-absorbable antibiotics as previously described (8), empiric or specific parenteral antibiotics (9), prophylactic platelet transfusions when the platelet count fell to $< 20{,}000/mm^3$, and granulocyte transfusions for infections not controlled by appropriate antibiotics alone.

RESULTS

Patient characteristics are listed in table 1. Of the 15 patients who received VAV, two patients had been treated earlier with 5-azacytidine in combination with methyl-GAG and one patient had achieved two PRs. Of the 14 patients who received AV, two patients had received VAV. Therefore, there were 29 patient trials for 27 patients entered in the study.

Two patients achieved a CR after one course of VAV. Consolidation therapy was not given but instead each received one maintenance therapy consisting of BCNU, 200 mg/m² by iv infusion, and cyclophosphamide, 1 g/m² by iv infusion, each drug to be repeated at 8 weeks (10). The maintained responses were short, lasting only 56 and 46 days. Reinduction was again attempted with VAV, and after two courses each, one CR and one PR were obtained. The CR patient then received a maintenance course of high-dose methotrexate, 50 mg/kg by iv infusion over 6 hours with a 72-hour folinic acid rescue to be repeated every month. Mucositis delayed the next course and the patient relapsed at 42 days. He subsequently received three courses of AV therapy without a response and died. The PR patient relapsed quickly (at 10 days) and failed to respond to another course of VAV. A course of daunorubicin, 60 mg/m²/day × 3, also failed and the patient died. No patient who received AV approached remission status. Survival was not different for either treatment group. All nonresponders proved to be refractory to any subsequent therapy and many died before another course of chemotherapy could be administered.

As expected, both combinations produced considerable hematologic toxicity. Median pretreatment wbc counts for the VAV and AV groups were 6000/mm³ (range, 800–17,000/mm³) and 14,800/mm³ (range, 300–76,000/mm³), respectively. The median nadir wbc counts were 1200/mm³ (VAV) and 700/mm³ (AV). The time to nadir was 9 (VAV) and 11 (AV) days. For responders the pretreatment median wbc count was 9700/mm³ (range, 1200–11,500/mm³) and the nadir wbc count was 800/mm³ (range, 400–1300/mm³). The time to nadir was 9 days. The duration of the nadir for all groups was 2–16 days. Platelet count suppression was marked in all patients. The median time to the nadir was 9–11 days and the median duration of the nadir was 10–16 days.

Nonhematologic toxic effects in both groups were comparable. Nausea and vomiting were universal but were severe in one patient in the VAV group and in five patients in the AV group. Most patients experienced some neuromuscular discomfort with weakness, lethargy, and occasional generalized muscle tenderness. Progression of the syndrome to irritability, confusion, and obtundation occurred in one patient shortly after finishing a first course of AV (after three prior courses of VAV) as a preterminal event in association with *Pseudomonas aeruginosa* sepsis. Severe pharyngitis and esophagitis occurred once in each group, and fever attributable only to the chemotherapy occurred once in the AV group.

DISCUSSION

In a previous study we demonstrated the activity of 5-azacytidine as a single agent. The present study asked if adding vinblastine and VP-16-213 would lead to a treatment with greater therapeutic effectiveness than 5-azacytidine alone. However, neither VAV nor AV produced a greater response in this study than 5-azacytidine alone in our previous study. When the CR rate achieved with 5-azacytidine alone in our previous study (five of 18) is compared with that of VAV in this study, the difference is not significant ($P = 0.3$). To date, > 80 patients with ANLL have been treated at our institution and elsewhere with 5-azacytidine in combination with other antileukemic agents as reviewed by Von Hoff et al (2). The numbers in each study have been small and the responses have ranged from 13% to 68%; these included the following combinations: 5-azacytidine + adenosine arabinoside, 14 patients with four CRs (29%) and no PRs;[3] 5-azacytidine + daunorubicin, 19 patients with 11 CRs (58%) and two PRs (10%);[4] 5-azacytidine + methyl-GAG, eight patients with no CRs and one PR (13%) (6); 5-azacytidine + adriamycin + bleomycin, one patient with one PR;[5] 5-azacytidine + cytosine arabinoside + prednisone + vincristine, 24 patients with 12 CRs (50%) and four PRs (17%);[4] and 5-azacytidine + β-2′-deoxythioguanosine, 19 patients with five CRs (16%) (11).

The toxic effects observed in this study were significant but were not considered unusual for any regimen containing 5-azacytidine in full dose. No difference was observed in toxic effects between the VAV and AV groups. The degree of leukopenia and bone marrow suppression was severe in both groups. In addition, the degree of myelosuppression between responders and nonresponders was similar.

It is disappointing that 5-azacytidine in combination with two other drugs with some activity in ANLL was not more therapeutically effective in this study. Recent studies of VP-16-213 in ANLL have not been as promising as initial reports (12). Nevertheless, because of the demonstrated activity of 5-azacytidine, clinical trials of different 5-azacytidine combinations are in order.

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Combination Chemotherapy With CCNU, Melphalan, Methotrexate, and Prednisone (CAMP) in 35 Patients With Advanced Breast Cancer [1]

Brief Reports and Preliminary Communications

Mogens Hansen,[2,*] Torben Palshof,[3] Kell Osterlind,[4] Heine H. Hansen,[2,3] and Henning Mouridsen [3]

During recent years, adjuvant chemotherapy for primary breast cancer has been found to delay the time to recurrences, particularly in premenopausal women (1,2). The purpose of this study was to investigate the effect of an orally administered combination, designed to cause minimal subjective symptoms from toxicity (particularly alopecia) with a minimum number of visits to the outpatient clinic.

MATERIALS AND METHODS

Therapy.—Each cycle consisted of (*a*) CCNU, 70 mg/m² orally on Day 1; (*b*) melphalan (Alkeran), 4 mg/m² orally on Days 1–5; (*c*) prednisone, 40 mg/m² orally on Days 1–5; and (*d*) methotrexate, 20 mg/m² orally on Days 22 and 25 (CAMP). This cycle was repeated every 6 weeks. Wbc and platelet counts were obtained on Days 1 and 22 of each cycle with dose modifications according to these cell counts. If wbc counts were between 2500 and 3500/μl and/or platelet counts were between 70,000 and 100,000/μl, the doses were reduced by 25%, but the dosage remained unchanged when counts were above these values. If the values were below the lower limits, the counts were followed weekly and treatment was withheld until both parameters were above these limits. Subsequently, the doses were reduced in the next cycle by 25% compared with the previous dose. However, patients with proven metastases to the bone marrow were treated regardless of platelet counts.

Patients.—A prerequisite for inclusion into the study was histologically proven breast cancer with progressive and measurable metastatic disease, ie, palpable metastases or radiologically measurable lung metastases. Patients were excluded if they previously had received chemotherapy. Radiotherapy to measurable lesions as well as oophorectomy or other endocrine therapy was not performed within 2 months before entry. The performance status according to Karnofsky should be >30. Finally, patients were asked to give their informed consent.

Parameters.—Prior to treatment, minimal evaluation included the following: clinical examination, hemoglobin, wbc and platelet counts, alkaline phosphatase, SGOT and LDH, serum calcium, serum creatinine, chest roentgenogram, and bone marrow examination. Hematologic status was controlled as mentioned above, while the other biochemical variables, clinical examination, and control of measurable lesions were performed at least once in each cycle.

Evaluation of results.—To be evaluable the patient had to receive at least one cycle of treatment. Complete response (CR) was defined as disappearance of all measurable lesions, and a partial response (PR) as at least a 50% reduction in the product of the diameters of measurable tumors without emergence of new lesions. Progression was defined as a 25% increase in measurable lesions or the appearance of new lesions. Reductions of $<50\%$ or increases of $<25\%$ within at least two series of treatment were considered no change. Malignant pleural effusions or bone metastases were not considered measurable lesions.

RESULTS

Thirty-five evaluable patients were included in the study from July 1975 to July 1976. Table 1 shows the patient age distribution and menopausal status (limit at 1 year after last menses) at the time of operation and of inclusion into the study. Twenty-seven of 30 patients had radiotherapy following primary surgery. Five patients with metastases at the time of surgery had no radiotherapy

[1]Received May 16, 1977; revised July 25, 1977; accepted Aug 11, 1977.

[2]Internal Medicine C, Bispebjerg Hospital, Copenhagen, Denmark.

[3]Department of Chemotherapy RII-RV, Finsen Institute, Copenhagen, Denmark.

[4]Internal Medicine B, Central County Hospital, Hillerød, Denmark.

**Reprint requests to:* Mogens Hansen, MD, Department of Chemotherapy RII-RV, Finsen Institute, Strandboulevarden, DK-2100 Copenhagen, Denmark.

TABLE 1.—Patient age and menopausal status, metastatic pattern, and responses (CR + PR) according to measurable lesions

Characteristic	At time of— Surgery	Entry to CAMP
Median age in yrs (range)	58 (38–75)	62 (43–78)
No. of patients—		
Premenopausal	6	1
Postmenopausal	29	34
No. of patients with evaluable metastases*	*CR*	*PR*
Soft tissue (28)		
Lymph nodes (24)	7	3
Cutaneous (10)	1	4
	8	7
Viscera (14)		
Lungs (13)	4	4
Thyroid gland (1)	0	1
	4	5
No. of patients with nonevaluable metastases*		
Bones (8)		
Pleura (11)		

*Nos. in parentheses = total No. of patients.

and entered the study within 2 months. The median time from primary operation to entry into the CAMP regimen for the other 30 patients with recurrences was 47 months (range, 2–156 months). Twenty patients had previously been treated for recurrences. For all 30 patients with recurrences the median time until first recurrence from the time of surgery was 29 months (range, 2–156 months). The distribution of metastases is shown in table 1.

The treatment results with the CAMP regimen are demonstrated in tables 1 and 2.

The average accumulated dose during the entire treatment period was 85% (range, 50%–100%) of the scheduled dose.

Toxic effects.—The frequency and grade of leukopenia and thrombocytopenia appear in table 3. Nine patients (26%) reported nausea and vomiting following the administration of drugs, mainly CCNU. Five patients (14%) developed stomatitis, in only one case severe. Two patients reported moderate symptoms from conjunctivitis, presumably caused by methotrexate. Mild alopecia was noticed in only one case. No patient died or refused treatment because of toxic effects.

TABLE 2.—Treatment results with CAMP in 35 patients with advanced breast cancer*

Response	No. of patients (%)	Median time (days) to response (range)	Median duration (wks) of response (range)	Median duration (wks) of therapy (range)	No. of patients on protocol at evaluation
Progression	5(14)			17(6–24)	0
No change	11(31)			18(8–42)	0
PR	8(23)	25(21–126)	18(6–44)	27+(12–53)	3
CR	11(31)	56(34–140)	19(9–31+)	29+(19–54)	3
Total CR + PR	19(54†)	42(21–140)	19(6–44)	29+(12–54)	6

*Results refer only to measurable lesions: soft tissue and visceral.
†95% confidence limits = 37%–71%.

TABLE 3.—Incidence of hematologic toxicity during treatment with CAMP

Wbc counts		Platelet counts	
Grade (cells × $10^3/\mu l$)	No. of patients (%)	Grade (cells × $10^3/\mu l$)	No. of patients (%)
> 3.0	20(57)	> 100	23(66)
3.0–2.0	9(26)	100–75	5(14)
2.0–1.0	5(14)	75–50	5*(14)
< 1.0	1(3)	< 50	2*(6)

*4 of these patients had bone metastases.

DISCUSSION

The drugs in the combination evaluated (CAMP) were chosen and the schedule was designed on the following presumptions: oral administration, few visits to the outpatient clinic, and a low grade of subjective toxic effects, including the avoidance of alopecia. The response rates obtained with each of these drugs alone were in the range of 12%–34% (3). Until recently, CCNU has been of little interest for the treatment of this disease (4); however, additional attention was paid to a probable synergistic effect between CCNU and melphalan (5).

The response rate and hematologic toxicity obtained with CAMP are comparable with results from trials with the most effective multiple-drug regimens (6). Although the duration of responses appears inferior, the low frequency of subjective symptoms from toxicity and the treatment and control requirements appear definitely in favor of the CAMP regimen.

The response rate achieved and the safety and ease of administration of CAMP qualify this regimen for controlled trials in advanced breast cancer in order to establish the reliability of its introduction into adjuvant studies.

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LETTERS

Prostatic Carcinoma Presenting as Pericardial Tamponade

Malignant pericarditis is a complication of several kinds of metastatic tumors, but is rarely seen even after autopsy of patients with prostatic carcinoma. Below we describe a patient whose first complaints were related to pericardial tamponade.

The patient was a 47-year-old man who was transferred from another hospital for evaluation of possible pericardial effusion. At the time of admission his blood pressure was 120/80 mm Hg, pulse rate was 100/minute, respiratory rate was 24/minute, and temperature was 37°C. A pulsus paradoxus of 40 mm Hg was noted. He was mildly dyspneic at rest. Auscultation of the chest revealed bronchial breath sounds below the angle of the left scapula. Marked jugular venous distention was present which did not decrease on inspiration. The cardiac impulse was diffuse; there was no murmur or rub. The liver span was 14 cm. The prostate was smooth and of normal size and consistency.

A chest roentgenogram showed cardiomegaly and segmental infiltrate in the right lower lobe. An electrocardiogram showed low voltage and electrical alternans. An echocardiogram confirmed the presence of a large pericardial effusion. Pericardiocentesis was productive of 1400 ml of sanguinous exudate with a wbc count of 4500/mm^3 (87% mononuclear). Cytologic study was consistent with adenocarcinoma. After pericardiocentesis significant hemodynamic improvement was noted. However, the subsequent hospital course entailed numerous complications and he died on his sixth hospital day.

At autopsy a segmental hemorrhagic infarct was found in the right lower lobe. A recent thrombus of the left circumflex coronary artery was present. The major finding was a pleomorphic, poorly differentiated adenocarcinoma of the prostate with metastases to the mediastinum. The primary tumor measured 3 × 1.5 × 1 cm. It was located posteriorly in the right lateral lobe and extended through the capsule near the ureterovesical junction. The mediastinum contained the only metastases found. An irregular, firm mass about 10 cm in diameter was adherent to the posterior wall of the left atrium. The pericardium contained about 500 ml of sanguinous fluid and was lined by a confluent sheet of tumor implants. Best's mucicarmine stains (to identify mucin-secreting cells) of both the primary tumor and the metastases were positive.

Carcinoma of the prostate is a common tumor of elderly men, and is capable of producing extensive metastatic disease. However, it uncommonly forms secondary tumors of the heart or pericardium (1–3). Even when present, the diagnosis is not made antemortem. Since this disease may be responsive to hormones or to newer forms of chemotherapy, the possibility of this complication should be considered by clinicians.

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Gerald V. Quinnan, Jr
H. Emerson Thomas, Jr*
Howard Sussman
Lawrence Kerzner

Departments of Medicine and Cardiology
Boston University Medical Center
University Hospital
75 E Newton St
Boston, Mass 02118

June 23, 1977

*To whom reprint requests should be addressed.

Autoregulation of L-Asparaginase-Induced Diabetes Mellitus[1]

The enzyme, L-asparaginase, has distinct utility in the treatment of acute lymphocytic leukemia (1). Unlike most antineoplastic agents, this drug possesses essentially no bone marrow toxicity; however, toxicity does occur, most notably abnormalities of liver function studies, plasma coagulation parameters, and immediate hypersensitivity reactions. On occasion, pancreatitis or overt diabetes mellitus may occur (1).

The combination of methotrexate (MTX) and L-

[1]Supported by Public Health Service grants CA-05138 and CA-08341 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. Dr. Capizzi is an Investigator of The Howard Hughes Medical Institute.

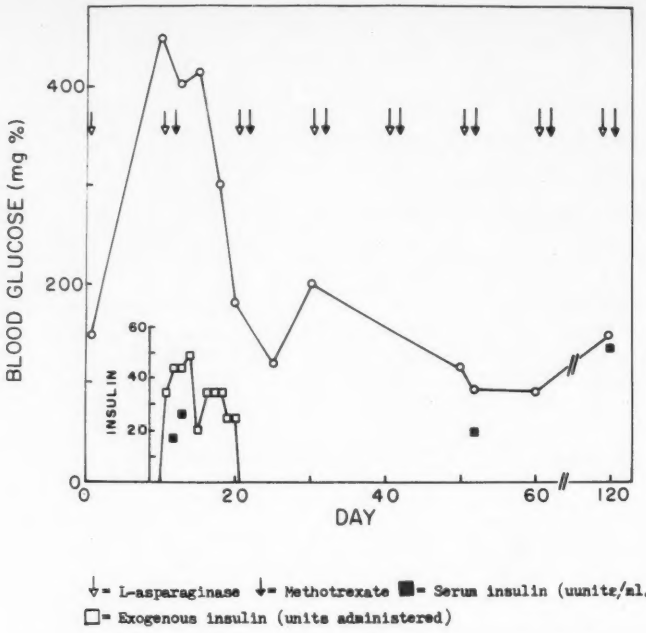

FIGURE 1.—Serial determinations of blood glucose and serum insulin. Glucose levels are fasting, except 2-hr postprandial levels on Days 1 and 120.

asparaginase is useful in the treatment of advanced acute lymphocytic leukemia (1). The following is a description of transient L-asparaginase-induced diabetes mellitus occurring in a 28-year-old moderately obese man with acute lymphocytic leukemia. The patient had relapsed after a 14-month remission during which time he received MTX, 6-mercaptopurine, and cyclophosphamide with intermittent vincristine and prednisone (2). Upon recognition of the relapse, therapy was begun with MTX and L-asparaginase. Prior to initiation of L-asparaginase therapy, his 2-hour postprandial blood sugar was 148 mg/100 ml. Ten days after 500 IU/kg of L-asparaginase, the fasting blood sugar was 458 mg/100 ml. Crystalline zinc insulin and neutral protamine Hagedorn insulin were administered to regulate the induced diabetes (fig 1). Serum insulin levels obtained during insulin treatment (blood drawn in the morning prior to next dose of insulin) were 18 microunits/ml on Day 11 and 26 microunits/ml on Day 12. There was no ketosis. He had no abdominal pain or tenderness and the serum amylase and lipase values were within normal limits. Despite continued intermittent therapy with MTX and L-asparaginase every 10 days, the exogenous insulin requirement decreased and was discontinued on Day 21. Remission was maintained for 8 months with MTX and L-asparaginase without elevation of the blood sugar levels above pretreatment values. Serum insulin on Day 120 was 56 microunits/ml with a 2-hour postprandial glucose of 148 mg/100 ml.

The diabetogenic effect of L-asparaginase has been noted previously in animals and man. The mechanism of the diabetes has been related to impairment of protein synthesis with decreased insulin production. Patients who have developed non-ketotic hyperglycemia due to L-asparaginase have been found to have very low serum-insulin levels. Pancreatic islet-cell damage is minimal or absent and hypoinsulinemia does not correlate with signs of pancreatitis. Hypotension secondary to the effects of intense hyperglycemia has been a cause of drug-related deaths (1). However, early recognition of the hyperglycemia permits adequate control with exogenous insulin.

The decrease in insulin synthesis occurs concurrently with a decrease in the synthesis of other proteins such as plasma coagulation factors. However, the plasma concentration of proteins which are synthesized in the liver returns toward normal despite continued treatment with L-asparaginase (1). This is possibly the result of induction of the

enzyme asparagine synthetase, which enables the cell to produce a sufficient concentration of the amino acid asparagine, thus resulting in renewed protein synthesis.

The use of L-asparaginase as a component of multiple-drug antileukemia protocols makes recognition of L-asparaginase-induced toxicity critical. The ability of various organs such as the liver and pancreas to adapt to L-asparaginase-induced impairment of protein synthesis suggests that it is not necessary to discontinue this potentially useful drug if appropriate supportive measures are undertaken.

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Barry Boston
Michael Rosen
Robert L. Capizzi*

Departments of Medicine and Pharmacology
Yale University School of Medicine
New Haven, Conn 06510

June 27, 1977

*Reprint requests to: R. L. Capizzi, MD, Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, NC 27514.

Guidelines for Training in Medical Oncology

The training program in medical oncology must provide advanced training that will make it possible for the physician to acquire the expertise to practice as a consultant in medical oncology. The program should not only provide training in the techniques required and fundamental clinical skills necessary for the practice of medical oncology, but should also provide an emphasis on scholarship, continuing self-instruction, development of critical judgment, and the ability to make appropriate decisions.

Medical oncology programs should be offered in institutions with residencies fully approved in internal medicine by the Liaison Committee for Graduate Medical Education. However, selected subspecialty programs of recognized excellence in institutions with an academic atmosphere but without a training program in internal medicine may be accepted. The minimum period of training for medical oncology is 2 years.

Medical oncologists must first be competent internists. The trainee in medical oncology should have 3 years of training in general internal medicine preferably prior to beginning training in the subspecialty. Throughout the clinical experience, learning in internal medicine should be continued. During the 2 years of training in medical oncology, at least 1 year should be an intensive clinical experience. Followup of patients throughout the 2-year program is necessary to enable the trainee to mature in the responsibilities of a consultant in medical oncology.

Program Director and Faculty

The program director should be certified in medical oncology or have the equivalent qualifications to provide adequate supervision of the trainees. Program directors are responsible for the adequacy of all facilities including support facilities, requisite to the provision of an education of high quality for the trainees. There should be sufficient individuals to constitute a training faculty. All members of the faculty are to be aware of the responsibilities of the program and accept the requirement for close, critical evaluation of the trainees' progress and competence.

Environment

Since the trainee in medical oncology must learn to integrate many scientific skills to prevent, detect, and manage neoplastic diseases, there must be, in the environment of the training program, broad support from other clinical specialties, especially diagnostic and therapeutic radiology, gynecology, pediatrics, nuclear medicine, pathology, and surgical subspecialties. In addition, it is desirable to have the support of oncologic nursing, rehabilitation, and social work. Medical oncology sections with responsibility for out-patient and in-patient care are essential for providing an appropriate training environment.

Specific aspects of allied specialties in which the trainee should develop a working knowledge include the following:

- *Diagnostic Radiology and Nuclear Medicine:* contrast studies and diagnostic techniques with radioactive isotopes; the reading of films with the radiologist in order to provide better feedback on clinical problems.
- *Pathology:* the importance of an integrative role in the evaluation of biopsy material between the pathologist and the needs of the patient; the various techniques for obtaining tissue for morphologic study; identification of neoplasms, recognition of abnormal cytology, and interpretation of morphologic findings.
- *Pediatric Oncology:* therapy for children with oncologic problems in cooperation with a pediatrician and/or cancer center.
- *Psychosocial:* the psychologic, sociologic, and spiritual needs of the patient.
- *Radiation Medicine:* the principles of radiobiology, types of equipment and indications for their use, and the results and limitations of radiotherapy.
- *Rehabilitation:* techniques designed to facilitate recovery and return to normal activity, including stomal care, physical therapy, laryngectomy voice training, prosthetics, and Reach to Recovery programs.
- *Surgery:* indications for surgery and decisions over major operative procedures in all major classes of tumors; familiarity with the results of surgical treatment, problems encountered during and following surgery.

Educational Program

The program must provide and the program director must document the presence of a sufficient number of new and followup patients to assure appropriate in-patient and out-patient experience with clinical neoplastic problems for each trainee. The principle of continuity of doctor-patient relationships during the period of training should be followed to the greatest possible extent, and a

specific out-patient clinic should be designated for followup care of medical oncology patients. The clinical experience should emphasize improvement in the history and physical examination. The learning process should assure skills in proctoscopy, pelvic examination, and head and neck examination.

The trainees should develop skill in functioning as primary care physicians. They should be able to establish a relationship with the individual or family and provide continuing surveillance of health needs, comprehensive care for the acute and chronic disorders for which they are qualified, and access to the health delivery system for disorders requiring the services of other specialists.

Conferences should augment learning by including clinical seminars conducted by the faculty, seminars reviewing subjects by the trainees, journal club meetings, and research conferences. Since the medical oncologist interacts with others to control or cure neoplastic diseases, interdisciplinary tumor conferences effectively demonstrate coordination of the best diagnostic and therapeutic methods. Attendance at pathology conferences, radiology conferences, and general medical conferences is encouraged with particular attention to those presenting problems relating to the diagnosis and management of neoplastic diseases.

To prepare for his role as a consultant to other specialists, the trainee should have opportunity to participate in a consultation team supervised by a faculty member. He should become experienced in teaching; specifically, the teaching should include medical students, house staff, and allied health personnel. The goal of these experiences should be an ability to correlate basic biomedical knowledge with clinical aspects of cancer and to integrate clinical management and teaching of cancer by all specialists at all stages of the disease.

The trainee should have an awareness of the importance of participation in postgraduate and/or continuing education and learn to provide public education in matters pertaining to cancer. The trainee should participate in the community system of health care relating to neoplastic diseases and work with all individuals in the health system.

The training program should have an investigational component. The trainee must develop a posture of alertness to the constant development of scientific progress and should be prepared to contribute to this aspect of the program. He should learn the design and interpretation of research studies, evaluation of investigative methods, and interpretation of data and develop competence in critical assessment of new therapies and the medical literature. An understanding of variations attributed to a disease, bias, criteria of measurement of tumor response, use of controls, factors in patient selection in clinical studies, and biostatistical techniques is essential. The research experience should include a clinical study in which the trainee reviews case records and assembles clinical data. He should learn the method of deriving a clinical protocol for research, develop one himself, and participate in research utilizing established protocols.

The organization of each program must include a mechanism for ongoing evaluation of the competence and progress of each trainee. Records of such evaluation should be maintained with appropriate counseling of each trainee.

Specific Knowledge and Skills

The principal skill of the medical oncologist is clinical judgment in matters relating to neoplastic diseases. The trainees should learn to quantify, measure, and define tumor growth. They will repeatedly apply skills from their general medical training including bone marrow aspiration/biospy, thoracentesis, paracentesis, lumbar puncture, proctoscopy, and the use of laryngeal mirrors. They must learn the indications for such procedures, how to interpret materials from such sources, and how to evaluate the results. Knowledge of basic science information is necessary for optimal management of the patient with cancer. Trainees must be well educated in the following subject matters and become accomplished in the procedures:

- *Biochemistry* (the basic concepts of molecular biology and cellular metabolism; specific metabolic pathways affected by drugs; the use of biochemical and immunochemical markers; the role of biochemical studies of serum, urine, and other fluids to evaluate the extent of the disease; biochemical alterations during progression of the disease and therapy; and the role of quantitative measurement of tumor cell products).
- *Biostatistics* (principles of biometry).
- *Cell Kinetics* (growth characteristics of normal and neoplastic cells including components of the cell cycle and mechanisms of cell division; concepts of growth fraction and cell loss; factors regulating cell growth; the concept of stem cells; characteristics of cell membranes; maturation and repair in normal and neoplastic cells; and the principles of chronology).
- *Chemotherapy* (the available drugs for treatment of neoplastic diseases and indications for their use in specific neoplasms; dosage schedules; techniques for drug administration, duration of therapy, and the results of therapy alone, in sequence, or in combination with

other drugs, and in combination with surgery and/or radiotherapy).

- *Clinical Pharmacology* (the locus, mechanisms of action, distribution, metabolism, cytokinetics, toxicity, and limitations of drugs used in cancer chemotherapy, including interaction with other drugs; clinical pharmacologic studies; therapeutic trials of new agents; broader trials of new agents demonstrated to be effective therapeutically; and the principles of study protocols and their use).
- *Cytogenetics* (karyotypic abnormalities in neoplastic diseases; methods and results of such chromosome analysis; effects of treatments with drugs and X-rays on chromosome morphology; and genetic factors in the development of neoplasia).
- *Endocrinology and Metabolism* (the endocrine manifestations of certain cancers; ectopic hormones; indications for hormonal therapy; management of patients following ablative endocrine procedures; the etiologies of weight loss; protein abnormalities; fever; and electrolyte disturbances).
- *Epidemiology* (the incidence of cancer with respect to race, sex, age, occupation, habits, and geography; the role of public health in community efforts for controlling cancer; and socio-economic factors).
- *Etiology* (the predisposing and causal factors leading to neoplasia including chemical carcinogens, viral oncogenesis, and occupational and environmental factors; and congenital and acquired diseases predisposing to neoplasia).
- *Hematology* (coagulation factors; diagnosis of the cause of bleeding, manifestations, and specific therapeutic indications; hematologic alterations from disease or cytotoxic drugs; metabolism, function, and fate of leukocytes; and familiarity of treatment with blood products including red blood cells, platelets, and granulocytes).
- *Hematopathology and Pathology* (details of peripheral blood and bone marrow examination; and ability to recognize the fundamental features of the histology of lymph node neoplasms and common cancers).
- *Immunology* (tumor immunology; pre-existent, co-existent, and resultant immunologic characteristics of host reactions in patients with neoplasms including cellular and humoral factors; and immunologic tests used in diagnosis and the current status of immunotherapy).
- *Microbiology* (the epidemiology, diagnosis, prevention, and treatment of infections; the use of prophylactic antibiotic therapy; and the role of protected environment techniques).
- *Natural History of Cancer* (the pattern of growth and spread of specific cancers in man; the expected duration of survival of the untreated patient; and the variabilities in the natural process of growth).

Editorial Comment

These guidelines for Medical Oncology Training Programs were prepared by the Medical Oncology Subspecialty Committee of the American Board of Internal Medicine. Future candidates should have completed 3 years of postgraduate training in the broad field of internal medicine. For certification in oncology, a minimum of 2 additional years is recommended, encompassing comprehensive training in the clinical management of the cancer patient. Particular emphasis is placed by the Subcommittee on diagnostic methods, familiarity with the natural history of various neoplasms, and skill in the use of chemotherapeutic agents. Continuity of patient care, a proper academic environment, and consultative experience are stressed. The program should also have an investigative component with opportunity for the candidate to engage in clinical and/or laboratory research.

The guidelines expressed in this document describe the ideal setting for oncology training, but these objectives are difficult to attain in most extant Medical Oncology Programs. The opportunity for exposure to a broad range of patients with cancer, the maintenance of continuity of care, consultative experience, and participation in clinical research are mutually exclusive in many institutions. For example, a commitment to protocol research necessarily narrows the range of neoplasms under study. Broad exposure necessitates rotation to various subspecialty areas, thus limiting continuity of care. Finally, thorough education in a formidable list of basic science disciplines (ranging through clinical pharmacology, endocrinology, immunology, etc) is unrealistic and would be superficial at best in most programs.

Medical oncology training might aim for less comprehensive objectives and concentrate on three basic areas: (1) a thorough knowledge of several common neoplastic diseases, including both hematologic malignancy and solid tumors, with emphasis on diagnosis, staging, natural history, and clinical management; (2) opportunity for exposure to a variety of tumor types with emphasis on principles of management; (3) an education program which stresses principles of oncology dealing with relevant aspects of cell biology (kinetics, biochemistry, immunology) as they relate to etiology, natural history and treatment; and (4) an opportunity to undertake

a research project. The latter area should stress investigative approaches to study and treatment of cancer, as well as the development of a critical ability to evaluate the literature and the application of research advances to the cancer patient.

As in most subspecialities, medical oncologists are differentiating along one of two major pathways. One is the route of private practice, where a broad range of practical knowledge in cancer diagnosis and management must be built upon a foundation of the principles of internal medicine. The other is an academic path, where investigative and educative skills must be stressed. Thus, the medical oncology candidate should seek a training program wherein his objectives can be most readily achieved. It will be the unusual institution which will fulfill all criteria set forth by the Subspeciality Committee.

ANNOUNCEMENTS

Faculty Position: Hematology/Oncology

The Section of Hematology and Oncology of the University of Arizona, College of Medicine, will have an opening available in 1978 for the appointment of a physician as an Assistant Professor of Medicine. The position is for addition to the faculty component based at the Tucson Veterans' Administration Hospital, which is a fully affiliated Dean's Committee Hospital of the University of Arizona.

Physicians with particular interest in an academic career and evidence of accomplishment in clinical trials, pharmacology, and related areas should apply. The academic appointment is with the University of Arizona, with salary support provided by the Veterans' Administration. Applicants should describe their areas of particular clinical and laboratory interest and activity, and include a copy of their curriculum vitae and bibliography, and names of three professional references.

Inquiries should be addressed to: Sydney E. Salmon, MD, Head, Section of Hematology and Oncology, University of Arizona, College of Medicine, Tucson, Ariz 85724. (The University of Arizona is an equal opportunity employer.)

Seminars in Oncology at Roswell Park Memorial Institute

Buffalo, New York

There will be a seminar on "Thyroid Carcinoma: Newer Concepts and Management" on January 26, 1978, organized by Department of Medicine B, Roswell Park Memorial Institute. The chairperson is Dr. Katsutaro Shimaoka.

There is a $10.00 registration fee. *For further information contact:* Ms. Claudia Lee, Cancer Control, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

Change in the Training Requirements for the American Board of Internal Medicine's Subspecialty Examinations

The American Board of Internal Medicine has announced a significant change in policy regarding its training requirements. This change will affect physicians desiring admission to a subspecialty examination who began their residency training in internal medicine before June 1, 1970. At present, these physicians are considered to have met the training requirements for admission to a subspecialty examination after completing 1 year of acceptable training in the subspecialty. If once informed of admission to a subspecialty examination, these physicians will continue to be considered to have fulfilled the Board's training requirements in that subspecialty. Beginning with the subspecialty examination offered in 1981, however, all physicians not previously admitted to an examination will be required to meet the same training requirements which apply to physicians beginning their residency training after June 1, 1970. These requirements currently involve 2 full years of acceptable training in the subspecialty.

A complete description of the Board's policies and procedures may be obtained by directing a written request to: American Board of Internal Medicine, 3930 Chestnut St, Philadelphia, Pa 19104. (The next examination in Medical Oncology will be offered in June 1979.)

Fellowships in Medical Oncology

Applications are now being accepted for fellowships in medical oncology at the University of Vermont and the Vermont Regional Cancer Center beginning July 1978 and July 1979. This program offers clinical and research activities in cancer chemotherapy, clinical and biomedical pharmacology, and tumor immunology. It includes extensive interdisciplinary emphasis in patient management and participation in national cooperative oncology groups to aid in the management of patients with solid tumors and hematologic malignancies.

Prerequisites include 2 years of approved medical residency and American Board of Internal Medicine eligibility. Candidates with academic interests are encouraged to apply.

Send informative letter and curriculum vitae to: Irwin H. Krakoff, MD, Director, Vermont Regional Cancer Center and Oncology Unit, University of Vermont, MCHV-Burgess, Burlington, Vt 05401.

XII International Cancer Congress

Buenos Aires, Argentina, October 5–11, 1978

The XII International Cancer Congress will be held in Buenos Aires, Argentina, October 5–11, 1978. Information, brochures, and registration forms are available by writing to Dr. Charles D. Sherman, Jr, Editor of the Newsletter of the American Federation of Clinical Oncologic Societies, University of Rochester Medical Center, 601 Elmwood Ave, Rochester, NY 14642. Registration *prior* to December 31, 1977, is $150.00; thereafter registration is $200.00.

Special travel programs based on greatly reduced air fares, which are otherwise unavailable, are also being offered and information concerning these programs are available from Dr. Sherman or from Garber Travel (official US agent for the congress) at 1406 Beacon St, Brookline, Mass 02146 (telephone, 617-734-2100).

4th Course on Medical Oncology

Genoa, Italy, January 18–21, 1978

The 4th Course on Medical Oncology, organized by the Italian Association on Medical Oncology in collaboration with the Oncological Institute of Genoa University and the Italian League Against Cancer (Genoa Section), will be held in Genoa, January 18–21, 1978, at the "Auditorium della Fiera del Mare."

The main topics will be: advances on cancer chemotherapy and interdisciplinary treatment of breast cancer, malignant non-Hodgkin's lymphomas, soft tissue sarcomas, lung cancer, gastrointestinal carcinomas, testicular carcinomas, bladder carcinomas, and cerebral tumors. The course will also deal with recent progress in childhood tumors.

The subscription fee is 80.000 Lit. (covering lunch and Proceedings of the course). *For further information contact:* Prof. Riccardo Rosso, Istituto di Oncologia della Università, Viale Benedetto XV 10, 16132 Genoa, Italy.

III Central American and Panamanian Oncologic Congress

Guatemala City, Guatemala, February 22–25, 1978

The III Central American and Panamanian Oncologic Congress will meet in the Hotel El Dorado Americana in Guatemala City on February 22–25, 1978. *For additional information, please write to:* Dr. Carlos Lizama Rubio, President, Instituto de Cancerología, 6a. avenida 6–58, zona 11, Guatemala City, Guatemala.

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